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MICROBIOLOGICAL PROFILE AND SAFETY OF FRESH JUICES SOLD IN THE CENTRAL BUS STATION AT KHARTOUM STATE

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ABSTRACT

Millions of people in developing countries especially in Sudan prefer to consume fresh juices. These types of juices are poorly prepared, handled and stored by street venders, hawkers and other food services under improper conditions. This may lead to disease outbreaks and other public health problems due to their possibility to transmit different diseases. Therefore this study was aimed to investigate the safety of fresh juices sold by vendors and hawkers. A total of 30 fresh juices of mango, guava, lemon, orange, fruit pulps including aradeib (*Tamarindus indica*), tabeldi (*Adansonia digitata*), gudeim (*Grewia* tenax), Shaeer or barely powder (*Hordeum vulgare*) and calyx of karkade (*Hibiscus sabdariffa*) were randomly collected from a central bus station at Khartoum for the investigation. The microbiological parameters studied were total viable bacterial count (TVBC), coliforms, yeasts and moulds using appropriate selective media. The TVBCs of all investigated samples exhibited counts in the range of $1.8x10^3$ - $3.0x10^6$cfu/ml. The count of total coliforms ranged from 3.4 to 1300MPN/ml. Yeasts were found in all samples in the range of $1.0x10^3$- $2.26x10^4$cfu/ml while moulds were found in 66.7% of the samples ranging from 0.00 to $9.0x10^3$cfu/ml. The dominant bacterial isolates were identified as *Staphylococcus aureus* (19.6%), *Bacillus cereus* (13.1%), *Bacillus subtilis* and *Streptococcus cremoris* (10.9%) each, *E. coli* (8.7%), *Streptococcus faecalis*, *Streptococcus thermophiles*, *Staphylococcus saprophyticus* and *Salmonella typhi* (6.5%) each, *Salmonella paratyphi* and *Staphylococcus epidermidis* (4%) each and *Salmonella orizonae* (2%). Moulds genera were identified as *Asperigillus* sp. (45%), *Rhizopus* sp. (32%) and *Penicillium* sp. (23%). Fresh Sudanese juices obtained from fresh fruits and pulps offered for sale are highly contaminated with spoilage and pathogenic microorganisms. Therefore they are considered as health threats for the consumers. Proper procedures, good manufacturing practices (GMP) and good hygienic practices (GHP) are recommended to improve the quality of fruit juices.

KEYWORDS: Sudan, Fresh Fruit Juices, Pathogenic Microorganisms, Safety, Spoilage Microorganisms.

INTRODUCTION

Juices are defined as an aqueous solution, purée of mature fruits or vegetables or any concentrate of such solution or purée from one or a mixture of fruits or vegetables.[1] Fresh juices are preferable by people of all ages worldwide due to their fresh flavor, high nutritional value, low price and availability anywhere. They are highly nutritious and provide consumers with vitamins and minerals.[2]

The production of juices either fresh or processed is older than agriculture.[3] They can be prepared by proper squeezing or pressing of edible mature fruits or by water extraction and can be frozen for later use. The use of low temperature is useful where it extends the shelf life of the juice by delaying spoilage microorganisms, viable pathogens growth and the indigenous enzymatic reactions. In Sudan fresh juices obtained from water extract are diluted due to their high acidity. Dilution is a common practice for too acidic or strongly flavourd juices to be pleasant for consumption.[4]

Most people considered that fresh juices are not hazardous for their health due to the natural acidic taste and strong flavor of the juices. Generally, many

pathogens can survive in acidic conditions and cause many foodborne illnesses.[5] There are many procedures for fresh fruit juices preparation during which many microorganisms will be introduced and contaminate the final product and thus lead to the product spoilage or food borne illness.

Contamination of fresh juices sold by vendors, hawkers, and other public services renders these products to be unsuitable for human consumption and has been considered as a health threats globally. Generally, fresh fruit juices will be contaminated from various sources such as raw materials, juice machine, handler and improper surrounding hygienic conditions.[6] The contamination of fresh juices with coliforms and other bacteria may be referred to the water sources.[7] Bacteria, yeasts and moulds in fresh fruits juices are the causative agent of food spoilage and diseases outbreak.[8] In many areas in the world there is a relation between the fruit juices consumption and food-borne illness.[9,10] The potentially hazardious bacteria in fresh juices are *E. coli* O157: H7, some species of *Salmonella*, *Shigella* and *Staphylococcus auresu*.[11] These pathogens are considered as a source of serious food poisoning outbreak.[12] More recent outbreaks of non-typhoidal salmonellosis in fresh juice have been referred to the fecal-associated contamination of fruit or poor processing practices.[13]

Khartoum is the most crowded city in Sudan and it is characterized by hot climate during a long summer season resulting in the increase of fresh juices consumption. In Sudan, the surveillance of food borne illness are not well documented.[14] These information are very important for the local authorities to control the production, establish health precautions and to deal with the violators. This research aimed to investigate the microbiological quality and safety of fresh juices sold at Jackson bus station which is considered as the main bus station in the center of Khartoum.

MATERIALS AND METHODS

Study area

Jackson yard is an area located south west Khartoum, the capital city of the Sudan. This yard is used as a station for buses coming from different Neighborhoods in Khartoum state. It is provided with several services such as water, rubbish bins, electricity. Several shops, cafeterias and stalls are located there for selling fresh juices, bottled water, soft drinks and sandwiches beside hawkers who vended different types of food including fresh juices. There is an improper disposal of their wastes due to insufficient rubbish collection bins in the area; therefore most of the people there dispose their waste on the streets.

Samples collection

A total of thirty fresh juice samples were randomly collected and purchased from juices dealers at Jackson bus station. These samples were prepared from different fresh fruits (mango, orange, lemon, guava, and mixed fruits), pulp of ardeeb (*Tamarindus indica*), tabeldi (*Adansonia digitata*), gudeim (*Grewia tenas*), calyx of karkade (*Hibiscus sebggrifia*) and Shaeer or barely powder (*Hordeum vulgare*). All collected samples were kept in sterile insulated iced containers and were immediately transported to the laboratory for microbiological investigation.

pH determination

The pH of fresh juice samples was analyzed by homogenizing 10 ml of the juice of each sample with 90 ml distilled water[15] using PYE model 211-pH meter (HANNA- pH 211- Portugal).

Microbiological parameters investigated

Preparation of serial dilution

Thirty ml of each sample were aseptically poured into sterile conical flask containing 270 ml of sterile peptone water and mixed well to obtain homogenous solution. Then ten-fold dilution was carried out as described by.[16]

Total viable bacterial count

Total viable bacterial count (TVBC) was conducted using the pour plate method.[16] From suitable dilutions 1 ml of each sample was transferred aseptically into sterile Petri dish, and 15ml of sterilized melted nutrient agar were added, mixed well, left to solidify and incubated at 37^{o}C for 2 days. Colonies were counted using a colony counter (Quebec Colony Counter) and the results were expressed as cfu/ml for each sample.

Coliforms and fecal coliforms determination

For total coliforms group determination the Most Probable Number (MPN) method was used. MacConkey broth and Brilliant Green Bile Lactose Broth (BGB) were used for presumptive and confirmatory tests respectively for coliforms presence.[17] The Most Probable Number (MPN) for both coliforms was reported using the MPN table.[17]

Yeasts and moulds count

Surface spreading technique was used to enumerate yeasts and moulds.[16] From suitable dilutions, 0.l ml was spread onto malt extract agar medium containing 0.1 g chloramphincol to suppress bacterial growth. Plates were incubated at 28^{o}C for 5 days. Colonies were counted using colony counter (Quebec Colony Counter). The results were expressed as cfu/ml for each sample.

Purification and identification of the microbial isolates

Predominant bacterial isolates were selected and sub-cultured onto nutrient agar medium. The purified isolates were then kept in refrigerator for further tests. Identification of bacterial isolates done by the conventional methods based on cultural, morphological and biochemical tests.[18,19,16] Yeasts isolates were not identified, while moulds isolates were identified to the

genus level using their morphological structure for each isolate.[20,21,22,23]

RESULTS AND DISCUSSION

Fresh juices either from fresh fruits or fruits pulp are very popular and favorable among people of all ages and people of different levels of life around the world particularly in Sudan. Due to this their safety and quality for the consumers and public health is very important.

Results shown in Fig.1. represents the pH of the collected fresh juice samples obtained from karkade, barley powder, fruits pulp of ardeeb, tabeldi and gudeim. The pH of the samples ranged between 2.7 and 5.5. Tamarind exhibited the lowest pH value, while barely powder showed the highest value ranging from 5 to 5.5.

With respects to the pH of juices obtained from fresh fruits, lemon juice samples recorded the lowest pH in the range of 2.23-2.60, while the other samples had a pH ranged from 3.23 to 4.6 (Fig. 2). The pH values of the fresh fruit juices in this study are lower than that obtained by[24] who found that the mean pH values of juices prepared from orange, sweet lemon and carrot were 4.34, 5.08 and 5.89 respectively and similar to that recorded by[25] who stated that the pH of processed juices sold in Ilorin Metropolis in Nigeria ranged from 2.40 to 3.79. Another study conducted by[26] claimed that the lemon juice samples had a pH of 2.3. Generally, the acidic nature of juices suppresses the growth of most bacteria and favours yeasts and moulds growth. In spite of that all juice samples in this research showed the growth of different bacteria, coliforms, yeasts and moulds.

The investigation revealed that all fresh juices samples had a microbial load ranged from log 3.3 to log 7.0 cfu/ml (Fig. 3 and 4). The highest TVBC (log 7cfu/ml) was recorded for the cocktail Juice samples purchased from stalls 11 and 12 (Fig.4) followed by the samples obtained from barely powder (log 6- 6.8cfu/ml) (Fig.3). Lemon juice samples showed the lowest TVBC ranged from log 3.3 to log3.4cfu/ml followed by orange juice samples obtained from stalls 3, 4 and 5 (log 3.5-log 3.7cfu/ml). The low bacterial count in these samples may be attributed to the low pH values of these samples which is similar to that found by[27] who referred the low bacterial load in grape juices to the low pH (3.94- 4.0). The TVBC of processed apple, mango and orange fruit juice in Pakistan ranged from log 3.2±0.29 to log 7.28±41cfu/ml.[28] The bacterial load of un-pasteurized packed fruit juices sold in Lahore city was reported to be log 6.8±1.91cfu/ml.[29] Papaya juices samples showed the highest total viable count (log 4.81cfu/ml), while grape juice revealed the lowest count (4.60cfu/ml) as confirmed by.[27] The homogenate street vended fruit salad in Calabar in Nigeria had microbial load ranging from log 5.54 to log 5.83cfu/g.[30] Cooked meal and juice samples sold in Atbara city of the Sudan were shown to be contaminated with bacteria of log 4.6cfu/g and log 4.1cfu/ml respectively.[31] Results obtained revealed that the TVBC of some investigated samples are within the permissible level (log 3cfu/ml) stated by[32] and less than (log5) recorded by[33] and[1] while some samples exceeded these levels.

The high TVBC values recorded in this study may be due to the effect of various factors. One major factor is the contamination of the fruits from the farm where they are exposed to contamination during harvesting, packaging, transportation, storage, and selling. The total viable count of fruits collected from different retail outlets ranged from log 6.11 to log 7.45cfu/g.[34] Generally, raw fruits and vegetables have been documented to harbor different pathogenic bacteria such as *Shigella* spp, *Salmonella* spp, enterotoxigenic and enterohemorrhagic *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter* spp, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, and parasites as *Giardia lamblia*, *Cyclospora cayetanensis* and *Cryptosporidium parvum*.[35] Spoilage and pathogenic microorganisms will be introduced into juices and lead to foodborne illness.[7] Subjection to mishandling by juice makers, poor personal and domestic hygiene, peeling of fruits before hands washing, dust particles and the ice used for cooling juices all these factors are considered sources of contamination thus increasing the TVBC of the final product.[36,37] Moreover, raw materials, equipment, poor knowledge about good manufacturing practices (GMP), and unhygienic surrounding conditions all these factors may increase the microbial load of the fresh juices prepared.[4]

Unavailability of running water for washing and dilution, poor and prolonged preservation of the juices without refrigeration increase the occurrence of outbreaks of food illness.[38,26] However, the sites of the juices building by the side of a busy road with heavy vehicular traffic containing airborne particles including dust or by the side of the waste disposal system and overcrowded area play an important role in the juices contamination.[39] Beside all these sources, the use of over ripened fruits, peeling and peeled fruits left for a long time exposed to different contamination sources are another potential threat to introduce microbiological hazards into the final product. However, dealing with money during serving of fresh juices to consumers is another source of contamination. Many researchers reported that money harbor different types of microorganisms as revealed by[40] who found that the South Sudanese pound exhibited high level of contamination with different microorganisms as coliforms, *E. coli* and *Staphylococcus aureus*. He revealed that this banknote plays a role in transmitting pathogens among food handlers and introduce these microorganisms into foods sold at the University of Juba restaurants.

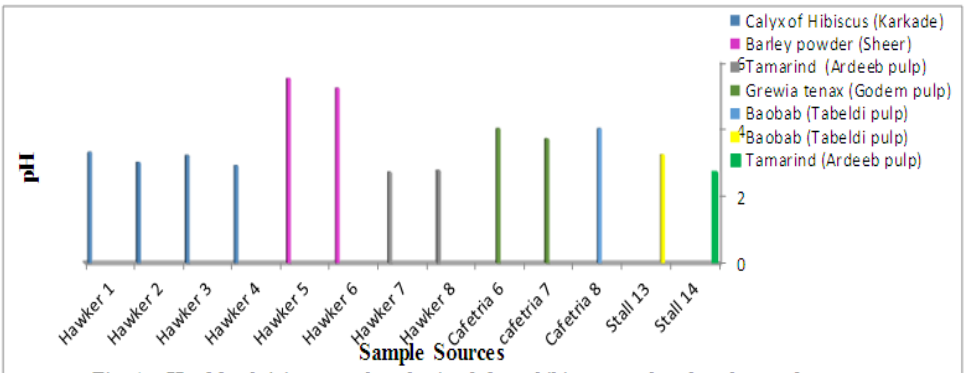

Fig. 1. pH of fresh juice samples obtained from hibisucus calyx, barely powder and differnt fruits plub.

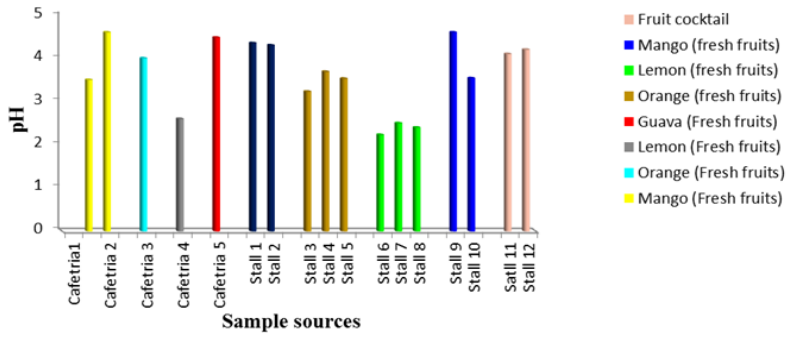

Fig. 2. pH of fresh juices obtained from different fresh fruits.

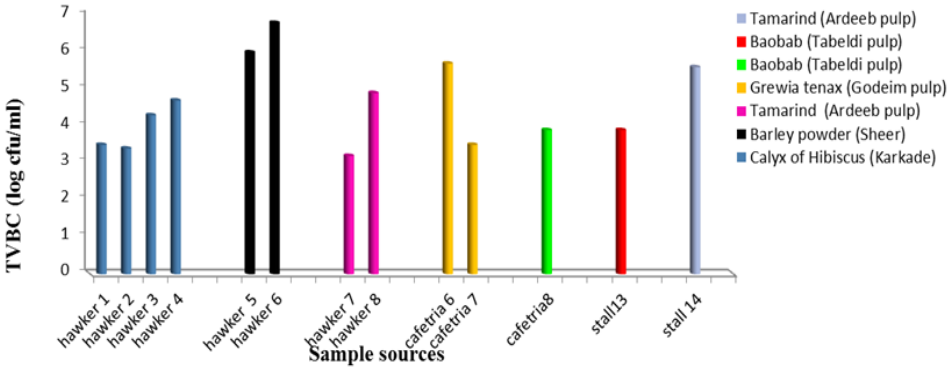

Fig. 3. Total viable bacterial counts of juice samples obtained from hubiscus calyx, barely powder and diffrent fruits pulp.

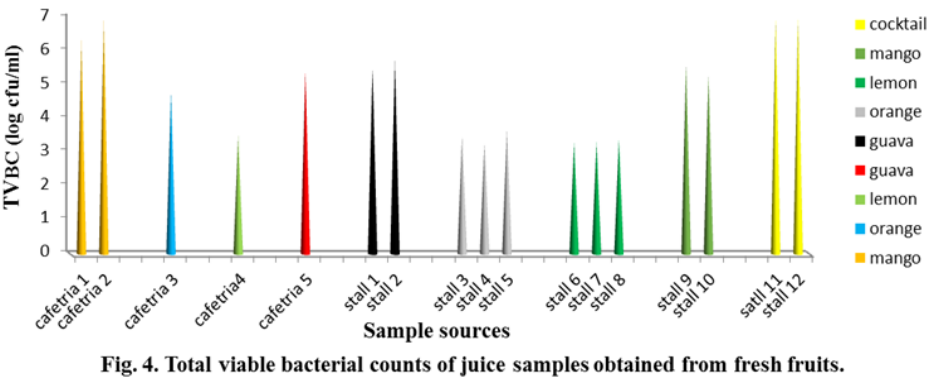

Fig. 4. Total viable bacterial counts of juice samples obtained from fresh fruits.

All Sudanese fruits and vegetables vendors display these products on the ground or in boxes for a long time, this prolonging exposure could leads to the entrance of microorganisms into the tissues (Plate 1, 2 and 3). Thus the ordinary way of washing is not efficient to eliminate the microbial hazards.

Generally, the total coliforms counts of the investigated samples recorded counts ranged from 3.4 to 1300MPN/ml as presented in Table. 1 and 2. Orange juice sample purchased from stall 3 exhibited a high count (1300MPN/ml) followed by mango juice obtained from cafeteria 3 (1100MPN/ml) and *Grewia tenax* juice (Gudeim pulp) from cafeteria 6 (1100MPN/ml). The total coliform count for sugar cane juice samples sold in Dhaka in Bangladesh ranged from log 2.76 to log 3.54 cfu/ml.[41] Another study reported by[28] who found that the total coliforms count of non- pasteurized packed juices sold in Lahore city in Pakistan ranged from 0.00 to log 4.48cfu/ml. Similar studies were conducted by.[42,43] They found that the total coliforms count of fruit juices was log 6.00 and log 4.56 cfu/ml respectively.

Results revealed that the total coliforms count in this study exceeded the maximum permissible level (log 2.0) established by the.[33,1] High prevalence of this bacteria indicates faecal contamination and the possibility of the presence of other pathogenic microorganisms which requires more control to obtain safe products.[44] The high incidence of coliforms in the investigated samples may be referred to the use of contaminated water, cross contamination from surfaces and utensils, surrounding area or from infected juice servers. The contamination of juices with *E. coli* might be through contaminated water supplies which used for utensils washing or to dilute juices.[45] Also[8] confirmed that the sterilized water used in the preparation of kunun drink samples did not show any coliforms presence.

Results claimed that coliforms, spoilage and pathogenic bacteria will grow in acidic medium of such juices. This indicated that these bacteria have a capability to survive in low pH. Survival of pathogens in acidic juices is attributed to their ability to regulate their internal pH and maintain it at neutral pH due to the combination of passive and active homeostasis force.[46] Adaptation of enteric bacteria in very low acid medium is referred to the enzymes induction which leads to the raising of internal pH and activates enzymes that devoted to the protection and repair of proteins and DNA.[47] Many researches documented that many pathogenic microorganisms can survive in fruit juices with low pH medium.[48]

Table 1: pH values and coliforms counts of the collected fresh juices samples obtained from hibiscus calyx, barely powder and fruits pulp.

Sample No	Juice Types	Sample Sources	pH	Coliforms MPN/ml
1	Calyx of *Hibiscus* (Karkade)	Vender 1	3.3	>161
2	Calyx of *Hibiscus* (Karkade)	Vender 2	3.0	150
3	Calyx of *Hibiscus* (Karkade)	Vender 3	3.2	> 161
4	Calyx of *Hibiscus* (Karkade)	Vender 4	2.9	100
5	Barley powder (Sheer)	Vender 5	5.5	4
6	Barley powder (Sheer)	Vender 6	5.0	15
7	Tamarind (Ardeeb pulp)	Vender 7	2.7	43
8	Tamarind (Ardeeb pulp) seeds	Vender 8	2.76	15
9	*Grewia tenax* (Gudeim pulp)	Cafeteria 6	4.00	1100
10	*Grewia tenax* (Gudeim pulp)	Cafeteria 7	3.70	>161
11	Baobab (Tabeldi pulp)	Cafeteria 8	4.00	3.3
12	Baobab (Tabeldi pulp)	Stall13	3.23	1100
13	Tamarind (Ardeeb pulp)	Stall14	2.70	280

Table 2: pH values and coliforms counts of the collected fresh fruit juice samples.

Sample No	Juice Types	Sample Sources	pH	Coliforms MPN/ml
1	Mango (Fresh fruits)	Cafeteria 1	3.5	11
2	Mango (Fresh fruits)	Cafeteria 2	4.6	15
3	Orange (Fresh fruits)	Cafeteria 3	4.00	1100
4	Lemon (Fresh fruits)	Cafeteria 4	2.60	70
5	Guava (Fresh fruits)	Cafeteria 5	4.48	92
6	Guava (Fresh fruits)	Stall1	4.35	0.00
7	Guava (Fresh fruits)	Stall 2	4.30	43
8	Orange (fresh fruits)	Stall3	3.23	1.00
9	Orange (fresh fruits)	Stall4	3.69	9
10	Orange(fresh fruits)	Stall 5	3.53	3.3
11	Lemon (fresh fruits)	Stall6	2.23	43

12	Lemon (fresh fruits)	Stall7	2.50	15
13	Lemon(fresh fruits)	Stall8	2.40	210
14	Mango (fresh fruits)	Stall9	4.60	150
15	Mango(fresh fruits)	Stall10	3.54	1100
16	Fruit cocktail	Stall 11	4.10	>1100
17	Fruits cocktail	Stall12	4.20	1100

Plate: 1

Plate: 2

Plate: 3

Yeasts counts of all samples ranged between log 3 and log 4.93 cfu/ml (Table. 3, 4), while moulds count ranged from 0.00 to log 4.73cfu/m. Results illustrated that 11 samples (33.3%) obtained from different juice sources (vendor 5, vendor 7, cafeteria 3, cafeteria 4, stall 3, stall 4, stall 8, stall 10, stall 11, stall 12 and stall 13) were free from moulds. According to[12] the fungal count of various brand of apple, mango and orange juice ranging from log 3.15 to log 5.23cfu/ml. Also[49] showed that the total fungal (Yeast/Moulds) counts ranged between log 1.59 and log 1.90 cfu/g for all vended samples (as breakfast and fresh fruit juices) ranging from 5.81to log 5.90cfu/ml for fresh fruit juices. As stated by [28] the fungal count ranged from log 1.75±0.58 to 4.40±0.20. Another similar research carried out by[30] showed that yeasts and moulds were detected in 50% and 70% of fruit salad samples sold in Calabar in Nigeria respectively. Generally, the yeast count was highest in pineapple juice followed by beetroot, mousambi, carrot, and grape juice respectively sold in Guntur, A.P in India.[50] The maximum permissible count of yeasts and moulds is log 3cfu/ml as reported by.[33,1]

Yeast and moulds are common contaminants in different food products. The presence of yeasts and moulds might be due to the favorable conditions for yeasts and moulds growth (sugars, acidic pH, aerobic conditions and abundant vitamins). Moreover the mixing of rottened fruits with the fresh one, unhygienic surrounding conditions, dust, dirty utensils, juice dealer's hands and the washing of cups in bucket or in one container. All these factors act as potential sources of contamination. Also their presence indicated the poor sanitary conditions during handling of fruits, juice preparation and extraction. Contamination of fruits surface with fungi explains the existence of these microorganisms during processing.[51]

Table 3: Yeasts and moulds of the collected fresh juice samples obtained from hibiscus calyx, barely powder and fruits pulp.

Sample No	Juices Type	Sample Source	Yeasts count (log cfu/ml	Moulds count (log cfu/mL
1	Calyx of *Hibiscus* (Karkade)	Vender 1	3.00	3.18
2	Calyx of *Hibiscus* (Karkade)	Vender 2	3.54	3.23
3	Calyx of *Hibiscus* (Karkade)	Vender 3	3.04	3.00
4	Calyx of *Hibiscus* (Karkade)	Vender 4	3.45	3.01
5	Barley powder (Sheer)	Vender 5	3.00	0.00
6	Barley powder (Sheer)	Vender 6	4.73	2.75
7	Tamarind seeds	Vender 7	3.00	0.00
8	Tamarind seeds	Vender 8	3.66	3.40
9	*Grewia tenax* (Godem Soaked seeds)	Cafeteria 6	3.78	3.73
10	*Grewia tenax* (Godem Soaked seeds)	Cafeteria 7	3.51	3.08
11	Baobab (Tabeldi Soaked seeds)	Cafeteria 8	4.04	3.40
12	Baobab (Tabeldi)seeds	Stall13	3.00	0.00
13	Tamarind (Ardeeb) seeds	Stall14	4.04	3.40

Table 4: Yeast and moulds counts of the collected fresh fruit juice samples.

Sample No	Juices Type	Sample Source	Yeast count (log cfu/ml)	Moulds count (log cfu/ml)
1	Mango (Fresh fruits)	Cafeteria 1	4.08	3.14
2	Mango (Fresh fruits)	Cafeteria 2	4.93	3.34
3	Orange (Fresh fruits)	Cafeteria 3	4.76	0.00
4	Lemon (Fresh fruits)	Cafeteria 4	3.90	0.00
5	Guava (Fresh fruits)	Cafeteria 5	3.78	4.73
6	Guava (Fresh fruits)	Stall1	3.20	3.00
7	Guava (Fresh fruits)	Stall 2	3.34	3.95
8	Orange (fresh fruits)	Stall3	3.18	0.00
9	Orange (fresh fruits)	Stall4	3.40	0.00
10	Orange(fresh fruits)	Stall 5	4.00	3.44
11	Lemon (fresh fruits)	Stall6	4.40	3.44
12	Lemon (fresh fruits)	Stall7	4.79	3.20
13	Lemon(fresh fruits)	Stall8	4.65	0.00
14	Mango (fresh fruits)	Stall9	4.08	3.41
15	Mango(fresh fruits)	Stall10	4.00	0.00
16	Fruit cocktail	Stall 11	4.54	0.00
17	Fruits cocktail	Stall12	4.00	0.00

Forty six bacterial isolates obtained by plating of the different collected juice samples were identified using different biochemical tests (Table 5). As observed from the results, 66.7% of the isolates were gram positive bacteria. *Staphylococcus aureus* accounted 19.6%, while 13.1% assigned to *Bacillus cereus*, 10.9% to each *Streptococcus cremoris* and *Bacillus subtilis*, 8.7% to *E. coli*, 6.5% to each *Streptococcus faecalis*, *Streptococcus thermophiles*, *Staphylococcus saprophyticus* and *Salmonella typhi*, 4% to each *Salmonella paratyphi* and *Staphylococcus epidermidis* and 2% to *Salmonella orizonae* (Fig. 5). The predominant bacteria in fresh juices were *Bacillus alvei, Bacillus subtilis, Bacillus polymyxa, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli* and *Enterobacter*.[29] The bacterial isolates obtained from the processed fruit juice samples sold in Nigeria were identified as *Bacillus* sp., *Micrococcus* sp, *Proteus* sp, *Escherichia coli* and *Lactobacillus* sp.[25] Different bacterial isolates obtained from street-vended pineapples, pawpaw, watermelons and coconut were identified as *Staphylococcus aureus*, *Micrococcus* sp., *Bacillus subtilis*, *Lactobacillus* sp., *Streptococcus* sp., *E. coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Serratia plymuthica*, *Serratia ficaria*, *Proteus mirabilis* and *Enterococcus faecalis* [52]. *Pseudomonas*, *Salmonella typhi*, *Shigella* sp., and *Streptococcus faecalis* isolated from raw mixed-vegetable salad sold in Accra, Ghana.[53]

The presence of different *Bacillus* sp. in collected juice samples are health threats for humans and considered as important causative agent of intoxication and food poisoning.[54] Their presence in such juice samples could be attributed to the unhygienic surrounding conditions, improper handling and also may be referred to their capability to form spores and survive adverse conditions, or originated from soil or plant equipment[28] or from juice ingredients.[55] The spoilage and pathogenic

microorganisms present in juice samples revealed the contamination which may be introduced from different sources as recorded by different researchers.\

The presence of *Streptococcus thermophiles*, *Streptococcus cremoris* and *Enterococcus faecium* in the studied fruit juice samples in this research is an indication that contamination may be originated from fruits, flowers, plant leaves and pulps. Yeasts and lactic acid bacteria are widespread and popular contaminants that were isolated from plant leaves, marula fruits flowers and pulps.[56] Therefore the prevalence of lactic acid bacteria in the investigated samples indicated that these fruit juices are promising sources of probiotics bacteria. The most common probiotics genera are *Lactobacillus* and *Bifidobacterium* and yeasts including *Saccharomyces cerevisiae* var. *boulardii*.[57]

Table 5: Identification of bacterial isolates of fresh juices samples collected from different sources.

Isolates No.	Isolates Code	Shape	Gram staining	Endo-spore staining	Motility	Aerobic growth	Anaerobic growth	Growth at 45°C	Growth in 10% Nacl	PH 9.5	Catalase	Oxidase	Acid from glucose	O/F	Urease	Indole	VP	Nitrate reduction	Arginine hydrolysis	Coagulase	Haemolysis	Tested sugars								Species
																						1	2	3	4	5	6	7	8	
1	HC	cocci	+	-	-	+	+	+	-	-	-	-	+	F	d	-	+	+	+	-	-	-	-	+	+	-	+	-	-	*Streptococcus cremoris*
2	HC	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
3	HC	rod	+	+	+	+	+	+	d	+	+	-	+	F	d	-	+	+	+	-	-	+	d	-	-	-	-	+	-	*B. cerseu*
4	HC	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
5	HC	rod	-	-	+	+	+	+	+	+	+	-	+	F	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	*E. coli*
6	HC	rod	-	-	+	+	+	-	+		-	+	+	F	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	*S. paratyphi A*
7	HC	rod	+	+	+	+	+	+	d	+	+	-	+	F	d	-	+	+	+	-	-	+	d	-	-	-	-	+	-	*B. cerseu*
8	TP	rod	+	+	+	+	+	+	-	-	+	-	+	F	+	-	-	+	+	-	-	+	+	d	+	+	+	+	d	*B. subtils*
9	TP	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
10	TP	cocci	+	-	-	+	+	+	-	-	-	-	+	F	d	-	+	+	+	-	-	-	-	+	+	-	+	-	-	*Streptococcus cremoris*

Legend:

(d) Delayed reaction. 1----------8: Sugar tested (HC) Hibiscus calyx juice.

(+) Positive reaction. 1- Sucrose. 5- Mannitol. (TP) Tamarind pulp juice.

(-) Negative reaction. 2-Cellobiose. 6- Raffinose. (VP) Voges-Proskauer.

(F) Fermentation. 3- Glactose. 7- Salicine. . (O/F) Oxidation fermentation test.

. 4- Mannose. 8- Xylose.

Con. Table 5: Identification of bacterial isolates of fresh juices samples collected from different sources.

Isolates No.	Isolates Code	Shape	Gram staining	Endo-spore staining	Motility	Aerobic growth	Anaerobic growth	Growth at 45°C	Growth in 10% Nacl	PH 9.5	Catalase	Oxidase	Acid from glucose	O/F	Urease	Indole	VP	Nitrate reduction	Arginine hydrolysis	Coagulase	Haemolysis	Tested sugars								Species
																						1	2	3	4	5	6	7	8	
11	TP	rod	+	+	+	+	+	+	d	+	+	-	+	F	d	-	+	+	+	-	-	+	d	-	-	-	-	+	-	*B. cerseu*
12	TP	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
13	TP	rod	-	-	+	+	+	+	+	+	+	-	+	F	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	*E. coli*
14	TP	cocci	+	-	-	-	+	+	+	+	-	-	+	F	+	-	+	+	+	-	-	+	-	+	+	+	-	+	-	*S. faecalis*
15	M	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
16	M	cocci	+	-	-	+	+	+	-	-	-	-	+	F	d	-	+	+	+	-	-	-	-	+	+	-	+	-	-	*Streptococcus thermophilas*
17	M	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. saprophyticus*
18	M	rod	-	+	+	+	-	+	+	+	-	-	+	F	-	-	-	+	-	-	-	-	-	+	+	+	-	-	+	*S. typhi*
19	M	cocci	+	-	-	+	+	+	W	+	+	-	+	F	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	*St. epidirmidis*
20	M	rod	-	+	+	+	-	+	+	+	-	-	+	F	-	-	-	+	-	-	-	-	-	+	+	+	-	-	+	*S. typhi*

Legend:

(d) Delayed reaction. 1----------8: Sugar tested (TP) Tamarind pulp juice.

(+) Positive reaction. 1- Sucrose. 5- Mannitol.. (M) Mango juice.

(-) Negative reaction. 2-Cellobiose. 6- Raffinose. (VP) Voges-Proskauer

(F) Fermentation. 3- Glactose. 7- Salicine. . (O/F) Oxidation fermentation test.

. 4- Mannose. 8- Xylose.

Con. Table 5: Identification of bacterial isolates of fresh juices samples collected from different sources.

Isolates No.	Isolates Code	Shape	Gram staining	Endo-spore staining	Motility	Aerobic growth	Anaerobic growth	Growth at 45°C	Growth in 10% Nacl	PH 9.5	Catalase	Oxidase	Acid from glucose	O/F	Urease	Indole	VP	Nitrate reduction	Arginine hydrolysis	Coagulase	Haemolysis	Tested sugars								Species
																						1	2	3	4	5	6	7	8	
21	G	rod	-	-	+	+	+	+	+	+	+	-	+	F	-	+	-	+	-	-	-	-			-	+	-			*E. coli*
22	G	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
23	G	rod	+	+	+	+	+	+	-	-	+	-	+	F	+	-	-	+	+	-	-	+	+	d	+	+	+	+	d	*B. subtils*
24	G	cocci	+	-	-	+	+	+	-	-	-	-	+	F	d	-	+	+	+	-	-	-	-	+	+	-	+	-	-	*Streptococcus thermophilas*
25	G	rod	-	-	+	+	+	-	+		-	+	+	F	-	-	-	+	-	-	-	-	-	-	=	-	-	-	-	*S. paratyphi A*
26	G	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. saprophyticus*
27	G	cocci	+	-	-	-	+	+	+	+	-	-	+	F	+	-	+	+	+	-	-	+	-	+	+	+	-	+	-	*S. faecalis*
28	L	rod	+	+	+	+	+	+	d	+	+	-	+	F	d	-	+	+	+	-	-	+	d	-	-	-	-	+	-	*B. cerseu*
29	L	cocci	+	-	-	+	+	+	-	-	-	-	+	F	d	-	+	+	+	-	-	-	-	+	+	-	+	-	-	*Streptococcus cremoris*
30	L	cocci	+	-	-	+	+	+	W	+	+	-	+	F	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	*St. epidirmidis*

Legend:

(d) Delayed reaction. 1----------8: Sugar tested (G) Guava juice.

(+) Positive reaction. 1- Sucrose. 5- Mannitol.. (L) Lemon juice.

(-) Negative reaction. 2-Cellobiose. 6- Raffinose. (O/F) Oxidation fermentation test.

(F) Fermentation. 3- Glactose. 7- Salicine. .

(VP) Voges-Proskauer 4- Mannose. 8- Xylose.

Con. Table 5: Identification of bacterial isolates of fresh juices samples collected from different sources.

Isolates No.	Isolates Code	Shape	Gram staining	Endo-spore staining	Motility	Aerobic growth	Anaerobic growth	Growth at 45°C	Growth in 10% Nacl	PH 9.5	Catalase	Oxidase	Acid from glucose	O/F	Urease	Indole	VP	Nitrate reduction	Arginine hydrolysis	Coagulase	Haemolysis	Tested sugars								Species
																						1	2	3	4	5	6	7	8	
31	L	rod	+	+	+	+	+	+	d	+	+	-	+	F	d	-	+	+	+	-	-	+	d	-	-	-	-	+	-	*B. cerseu*
32	L	rod	-	-	+	+	-	+	-	-	+	-	+	F	-	-	-	+	+	-	-	+				-				*S. arizonae*
33	L	rod	+	+	+	+	+	+	-	-	+	-	+	F	+	-	-	+	+	-	-	+	+	d	+	+	+	+	d	*B. subtils*
34	Cock	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
35	Cock	cocci	+	-	-	+	+	+	-	-	-	-	+	F	d	-	+	+	+	-	-	-	-	+	+	-	+	-	-	*Streptococcus cremoris*
36	Cock	rod	-	+	+	+	-	+	+	+	-	-	+	F	-	-	-	+	-	-	-	-	-	+	+	+	-	-	+	*S. typhi*
37	Cock	rod	+	+	+	+	+	+	d	+	+	-	+	F	d	-	+	+	+	-	-	+	d	-	-	-	-	+	-	*B. cerseu*
38	Cock	cocci	+	-	-	+	+	+	-	-	-	-	+	F	d	-	+	+	+	-	-	-	-	+	+	-	+	-	-	*Streptococcus thermophilas*
39	Cock	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
40	Cock	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. saprophyticus*

Legend:

(d) Delayed reaction. 1----------8: Sugar tested (Cock) Cocktail juice.

(+) Positive reaction. 1- Sucrose. 5- Mannitol.. (L) Lemon juice.

(-) Negative reaction. 2-Cellobiose. 6- Raffinose. (O/F) Oxidation fermentation test.

(F) Fermentation. 3- Glactose. 7- Salicine. .

(VP) Voges-Proskauer 4- Mannose. 8- Xylose.

Con. Table 5. Identification of bacterial isolates of fresh juices samples collected from different sources.

Isolates No.	Isolates Code	Shape	Gram staining	Endo-spore staining	Motility	Aerobic growth	Anaerobic growth	Growth at 45°C	Growth in 10% Nacl	PH 9.5	Catalase	Oxidase	Acid from glucose	O/F	Urease	Indole	VP	Nitrate reduction	Arginine hydrolysis	Coagulase	Haemolysis	Tested sugars								Species
																						1	2	3	4	5	6	7	8	
39	Cock	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
40	Cock	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. saprophyticus*
41	O	rod	+	+	+	+	+	+	-	-	+	-	+	F	+	-	-	+	+	-	-	+	+	d	+	+	+	+	d	*B. subtils*
42	O	cocci	+	-	-	-	+	+	+	+	-	-	+	F	+	-	+	+	+	-	-	+	-	+	+	+	-	+	-	*S. faecalis*
43	O	cocci	+	-	-	+	+	+	+	+	+	-	+	F	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	*St. aureus*
44	O	rod	-	-	+	+	+	+	+	+	+	-	+	F	-	+	-	+		-	-	-			-	+	-			*E. coli*
45	O	rod	+	+	+	+	+	+	-	-	+	-	+	F	+	-	-	+	+	-	-	+	+	d	+	+	+	+	d	*B. subtils*
46	O	cocci	+	-	-	+	+	+	-	-	-	-	+	F	d	-	+	+	+	-	-	-	-	+	+	-	+	-	-	*Streptococcus cremoris*

Legend:

(d) Delayed reaction. 1----------8: Sugar tested (Cock) Cocktail juice.

(+) Positive reaction. 1- Sucrose. 5- Mannitol.. (O) Orange.

(-) Negative reaction. 2-Cellobiose. 6- Raffinose.

(F) Fermentation. 3- Glactose. 7- Salicine. .

(VP) Voges-Proskauer 4- Mannose. 8- Xylose.

(O/F) Oxidation fermentation test.

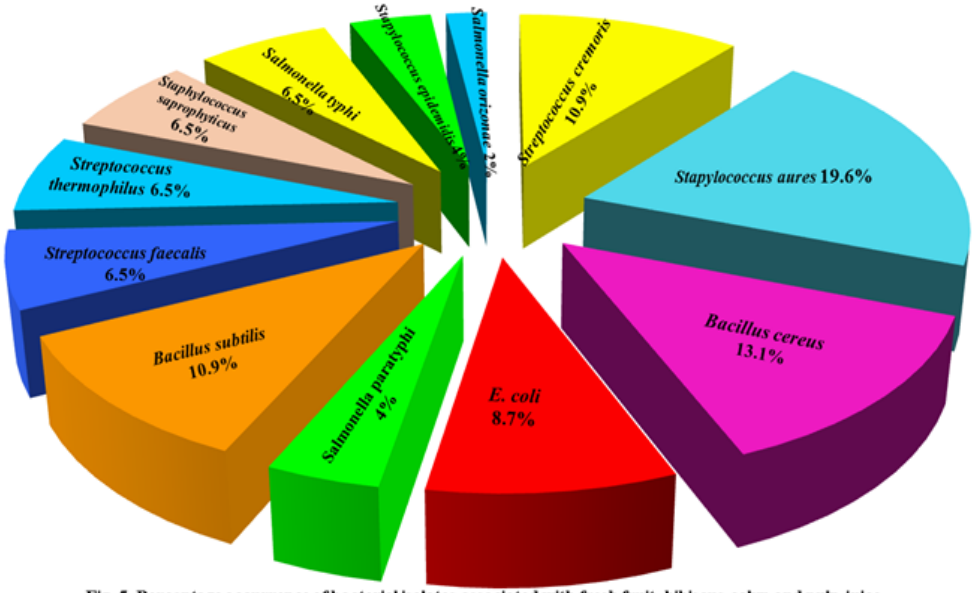

Fig. 5. Percentage occurrence of bacterial isolates associated with fresh fruit, hibiscus calyx and pulp juice samples purchased from different sources.

The predominant moulds isolated from the tested samples were identified as *Asperigillus* sp. (45%), *Rhizopus* sp. (32%) and *Penicillium* sp. (23%). The incidence of moulds in different fresh fruit juice samples had been reported by.[25] He isolated 4 genera isolated 4 genera which were identified as *Aspergillus nidulans*, *Aspergillus niger*, *Cladosporum* sp. and *Schizosaccharomyces pombe*. As stated by[24] *Alternaria* sp., *Aspergillus terreus*, *Aspergillus niger*, *Cladosporium*, and *Fusarium* were observed in tested juice samples. *Asperigillus* species are areal contaminants and can be isolated from different food products.[58] Moulds are associated with fruit juices spoilage by changing their taste or producing stale off–flavour or loss of juice cloud. Among these some moulds produce toxins which affect human and animal health. The major types of mycotoxins produced by some moulds are byssochlamic acid produced by *Byssochlamys fulva* and *B. nivea*, patulin excreted by *B. fulva*, *B. nivea*, ochratoxin by *Aspergillus carbonarius*, and citrinin which is produced by *Penicillium expansum* and *P. citrinum*.[24] As it can be noticed from the results all fruit juice samples in this study harbor many types of spoilage and pathogenic microorganisms which have been introduced from different sources. The presence of pathogenic microorganisms is health threats for human health and considered as an important causative agent of intoxication and food poisoning. These findings are alarming signs to set some decisions, health precautions and legislations by health authorities for juices preparation and manufacturing to prevent potential hazards as pathogenic microorganisms and to find natural plant preservatives as mint, ginger and beneficial microorganisms to control spoilage and pathogenic microorganisms in juices.

Therefore to obtain a product with very low microbial load, raw materials used for juices production should be of high quality. Careful handling of juices during processing and preparation should be maintained. Personal hygiene should be followed beside the implementation of proper GMP. Maintenance of adequate chill temperatures during selling would improve the microbiological quality and enhance the shelf life of the juices.

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