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Original Research Article

Microbial quality of herbal juices sold at sport complex in Washim

Nikhilesh Kulkarni*, Jayshree Narwade, Rachana Pachori, Prithviraj Sadar

Department of Microbiology, R. A. College, Washim, Maharashtra, India

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***Correspondence:**
Dr. Nikhilesh Kulkarni,
E-mail: drnskulkarni29@gmail.com

ABSTRACT

Background: Herbal juices are gaining global attention due to their medicinal properties, nutritive and antioxidant activity. They are frequently consumed by the people of all age groups in the form of health drinks. However, many outbreaks of human infections have been reported to be associated with the consumption of contaminated herbal juices. Hence, microbial quality assessment of herbal juices is utmost important.
Methods: The present study was undertaken to detect the existence of enteric pathogens as well as coliforms in the herbal juices sold at sport complex in Washim city area. The antimicrobial susceptibility testing was also performed to evaluate the MDR status of the isolates.
Results: The results showed that most of the herbal juices are contaminated with *Salmonella, Shigella* and *Coliforms*. The pathogens were found to be multiple drug resistant strains which pose an alarming threat for the consumers.
Conclusions: Regular monitoring of the quality of herbal juices for human consumption is recommended to avoid disease outbreak.

Keywords: *Coliforms*, Enteric pathogens, Herbal juices, *Salmonella, Shigella*

INTRODUCTION

Herbal juices are becoming an important part of the modern diet in many communities.[1] They are important dietary source of vitamins, minerals, fiber and other natural substances. Consumption of herbal juices provides potential health benefits to the general population viz. reduces the risk of chronic diseases and cancers, prevent vitamin deficiencies, reduces blood cholesterol level, maintains blood lipid profile as well as detoxify human body.[2-4]

Fresh herbal juices are well recognized for their nutritive value, mineral and vitamin content.[3] The health authorities had recommended consuming herbal juices daily as a part of diet. However, the outbreaks of food borne illness associated with the consumption of herbal juices are frequently reported all over the world.[5,6]

The herbal juices generally get contaminated by improper handling, Contaminated raw materials and equipment, unhygienic conditions of the personal, water used for the blending of fruits and vegetables also the pathogenic organisms can enter fruits and vegetables through damaged surfaces viz. punctures, wounds, cuts and splits that occur during growing or harvesting.[7,8]

Such juices have shown to be the potential sources of bacterial pathogens viz. *E. coli* O157:H7, *Salmonella* and *Shigella* species, *Staphylococcus aureus*, *Vibrio cholera*.[9,10] making them unacceptable for human consumption and thus become a global health problem.[11]

Hence, taking into consideration the high demand for fresh juices and safety of the consumers as well as prevention of disease outbreak due to contaminated herbal juices, the present study entitled "Microbial

Quality of herbal Juice Sold at Sport Complex in Washim" has been undertaken.

METHODS

Collection of juice samples

Total six different samples of herbal juices viz. Aloe Vera, Bitter gourd, Bottle gourd, Bael, Neem and Neem bark juice were purchased and collected in sterilized BOD bottles from the sport complex located in Washim city area. The collected samples were immediately transported to Microbiology research laboratory, R.A. College Washim for further investigation.

Microbiological quality assessment of collected juice samples

The collected herbal juice samples were further subjected for microbiological quality assessment adopting various techniques viz. standard plate count for bacterial load, most probable number (MPN) for coliforms as well as screening for enteric pathogens followed by their isolation and identification by the standard conventional methods.[12]

Determination of antibiotic susceptibility of isolates

The isolated pathogens were subjected to antibiotic sensitivity test by adopting disc diffusion technique.[13] Total twenty routinely recommended antibiotics viz. Ampicillin (10mcg), Azithromycin (15mcg), Chloramphenicol (30mcg), Ceftriaxone (30mcg), Ofloxacin (5mcg), Tetracycline (30mcg), Amoxicillin (10mcg), Cefotaxime(30mcg), Ciprofloxacin (5mcg), Gentamicin (10mcg), Co-trimoxazole (25mcg), Imipenem (10mcg), Co-amoxiclav (30mcg), Ceftazidime (30mcg), Nitrofurantoin (300mcg), Nalidixic (30mcg), Streptomycin (10mcg), Aztreonam (30mcg), Trimethoprim (5mcg), Norfloxacin (10mcg) were used for antibiotic susceptibility test.

RESULTS

Standard plate count of herbal juices

Total six different herbal juice samples viz. Aloe Vera, Bitter gourd, Bottle gourd, Bael, Neem and Neem bark juice were analyzed for its microbiological quality. The standard plate count was determined separately for each sample. The results are presented in Table 1. From the result it was observed that, in case of Bottle guard juice the highest SPC was recorded as (472cfu × 10^4/ml) followed by Aloe Vera (442cfu × 10^4/ml), Bael (142 cfu × 10^4/ml), Neem juice (126cfu × 10^4/ml), Bitter gourd (112 cfu × 10^4/ml) respectively.

The lowest SPC of (112 cfu × 10^4/ml) was recorded in neem bark juice. Hence, Bottle gourd juice was found to be the most contaminated juice whereas lowest contamination was observed in Neem bark juice. However, almost all the herbal juices were found to be possess bacterial contamination as compared to control which indicates the risk of acquiring infections in human consuming the juices for their health benefits.

Table 1: Standard plate count of herbal juices.

Name of juice	Spc/ml (cfu× 10^4/ml)	Control (sterilized juice)
Bottle gourd	472	00
Bitter gourd	112	00
Neem	126	00
Aloevera	442	00
Neem bark	88	00
Bael	142	00

Most probable number of herbal juices

Table 2 represents the findings on MPN of herbal juices.

Table 2: MPN of herbal juices.

Name of juice	3 of 10ml each	3 of 1ml each	3 of 0.1ml each	Mpn/100ml	Remark * p/np
Bottle gourd	1	0	1	7	NP
Bitter gourd	2	0	1	14	NP
Neem	1	0	0	4	NP
Aloe Vera	2	1	0	15	NP
Neem bark	0	1	0	3	NP
Beal	2	2	1	28	NP
Control (sterilized juice)	0	0	0	0	P

*P =potable NP= not potable

It was observed that the bael juice showed high MPN per 100 ml of (28) followed by Aloe Vera (15), Bitter guard (14), Bottle guard (7) and in case of Neem and neem bark juice, it was only (4) and (3) per 100 ml respectively. The

results exposed that the maximum faecal contamination was present in Bael juice and the lowest contamination was observed in Neem bark juice.

These results indicated that almost all the juices were contaminated with fecal coliforms and seem to be non-potable; However, the Bael juice may be at higher risk of having the human pathogens as well as may be most complimentary for bacterial growth.

Screening for the enteric pathogens in herbal juices

Table 3 represents the results on the screening for the enteric pathogens in herbal juices. From the result it was observed that, almost all the herbal juice samples analyzed showed the presence of enteric pathogens. In case of Bottle guard, Bitter guard, Aloe Vera and Bael juices both *Salmonella and Shigella species* were isolated. However, in Neem juice only *Shigella species* was detected. While, in case of Neem bark juice both the pathogens were not screened out indicating their absence in the juice. Hence, almost all the herbal juices showed the presence of enteric pathogens except Neem Bark juice in which both the isolated pathogens were not evident.

Table 3: Screening for the enteric pathogen in different juices.

Name of juice	*Salmonella Sps*	*Shigella sps*	Control (sterilized juice)
Bottle gourd	Present	Present	Absent
Bitter gourd	Present	Present	Absent
Neem	Absent	Present	Absent
Aloe Vera	Present	Present	Absent
Neem bark	Absent	Absent	Absent
Bael	Present	Present	Absent

Determination of antibiotic susceptibility of isolates

Total 02 enteric pathogens isolated from different herbal juice samples viz Aloe Vera juice, Bitter gourd juice, Neem bark juice, Neem juice, Bael juice and Bottle gourd juice were analyzed for its drug response. Table 4 represents the findings on the antibiotic susceptibility testing of isolates. From the table it was observed that the isolated pathogens showed the wide range of differences in their susceptibility pattern to the tested antibiotics. The results indicated that both the isolated pathogen viz. *Salmonella* and *Shigella* species were highly sensitive to Azithromycin, Amoxicillin, Cefotaxime, Gentamycin, Co-trimoxazole, Ceftazidime and Aztreonam. In case of Ampicillin, Ofloxacin and Imipenem, *Shigella* species showed sensitivity while S*almonella species* was found to be sensitive against only streptomycin. While, both isolated pathogens were found to be Intermediate against Chloramphenicol, Ceftriaxone, Nitrofurantoin, Nalidixic acid and Norfloxacin. In case of antibiotics, Ampicillin, Tetracycline, and Imipenem Only *Salmonella* species was Intermediate whereas *Shigella* species was found to be intermiadiate to Co-amoxiclave, Streptomycin and Trimethoprim. Both *Salmonella* and *Shigella* species were found to be resistant to Ciprofloxacin. In case of Ofloxacine, Co-amoxiclave and Trimethoprim, *salmonella* species showed resistant whereas *Shigella species* was resistant to only Tetracycline. Hence, *Salmonella species* showed 40% and *Shigella species* showed 50% sensitivity against the tested antibiotics while resistance per cent for *Salmonella species* was 20 and *Shigella species* was found to be 10% respectively. The intermediate status of both *Salmonella* and *Shigella species* was found to be at par (40%).

Table 4: Determination of antibiotic susceptibility of isolates.

Antibiotics	Drug concentration	*Salmonella Species*	*Shigella species*
		Zone of inhibition in (mm)	
Azithromycine	15 ug	22 (S)	22 (S)
Ampicillin	10 ug	15 (I)	17 (S)
Amoxicillin	10 ug	23 (S)	21 (S)
Chlorophinicol	30 ug	15 (I)	16 (I)
Ceftriaxone	30 ug	18 (I)	18 (I)
Tetracycline	10 ug	18 (I)	12 (R)
Ofloxacin	10 ug	18 (R)	6 (S)
Cefotaxime	30 ug	19 (S)	21 (S)
Ciprofloxacin	5 ug	8 (R)	10 (R)
Gentamicin	10 ug	32 (S)	24 (S)
Co-trimoxazole	25 ug	19 (S)	22 (S)
Imipenem	10 ug	15 (I)	16 (S)
Co-amoxiclav	30 ug	14 (R)	14 (I)
Ceftazidime	30 ug	19 (S)	20 (S)
Nitrofurantoin	300 ug	16 (I)	14 (I)
Nalldixic	30 ug	14 (I)	17 (I)
Streptomycin	10 ug	16 (S)	14 (I)
Aztreonam	30 ug	16 (S)	20 (S)
Trimethoprim	5 ug	10 (R)	11 (I)
Norfloxacin	10 ug	16 (I)	14 (I)
Sensitivity (%)		40	50
Resistance (%)		20	10
Intermediate (%)		40	40

DISCUSSION

The fruits or vegetables are contaminated with human pathogens by way of naturally active or passive mode of transmission, as well as the juicing method and unhygienic juice handling put into practice put forward that, still despite the fact the juice consumption is advantageous for the human health, However, the juice may be the probable source of human pathogens particularly for gastrointestinal tract and may outcomes in an occurrence of the epidemic diseases.

In Washim city, a huge section of the population consume fresh pressed and squeezed juices, most of these juices are sold by street vendors. The present study allowed to free-thinking that most of the herbal juices sold in this area contain significant bacterial counts. Improper washing of vegetables and fruits add these

bacteria to extracts leading to contamination. In addition, use of unhygienic water, preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. In the present study herbal juices have shown to be potential sources of bacterial pathogens notable species of *Salmonella, Shigella* and coliforms. The presence of coliforms in the fruits juice is indicative of fecal contamination. Improperly prepared fresh herbal juices may predictable as an emerging cause of food borne illness. Our findings are similar with the findings of other researchers working on the same line of research.[14] They reported the microbial contamination in street vended juices due to poor hygienic quality and the possible risk to consumers. Similarly, other researchers had reported high MPN in fruit juices as well as water samples used for blending the raw material.[15] Improperly prepared fresh fruits and vegetable juices are recognized as an emerging cause of food borne illness.[16]

Many researchers had reported the outbreaks of food borne illnesses associated with the consumption of herbal juices at several places in India and elsewhere.[17-20] Foods and beverages prepared and sold by street vendors have contributed to transmission of cholera and enteric diseases in Latin America. Cholera transmission was associated with consumption of street-vended beverages in Peru, Thailand.[21] Food borne disease outbreaks from enteropathogenic bacteria viz. *Salmonella, Vibrio cholerae, Vibrio parahaemolyticus* and *Staphylococcus aureus* are frequently reported throughout the world.[22] Number of studies on knowledge and practices of street food vendors regarding food safety conducted in different countries have shown a gap between knowledge on food hygiene and handling practices.[23-25]

CONCLUSION

The results of the present findings clearly demonstrated that, the road side ready herbal juices did not meet public health standards and many kinds of entero pathogenic bacteria were found namely, *Escherichia coli, Salmonella* and *Shigella species.* Such juices lead to hazardous effects to the consumers. Government agencies must adopt measures to educate the vendors about food safety and hygienic practices and enforce adequate guidelines with the help of Microbiologist, for juice preparations, especially street vended fruit juices.

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