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Antimicrobial action of essential oils against food borne pathogens isolated from street vended fruit juices from Baripada Town, India

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Abstract

The aim of this work was to study the presence of food borne pathogens (Bacteria and Yeasts) in street vended fruit juices and to investigate the antimicrobial activity of ten essential oils, for a potential use in food industries. Forty one samples of four different types (Orange, Grapes, Mosambi and Sugarcane) of fruit juices were collected from vendors following standard practices. Presence of high aerobic bacterial load was observed in the samples. Sixty percent of the samples were positive for presence of coliforms. Pathogenic bacteria like Arizona sp., Bacillus sp., Escherichia coli, Enterobacter sp., Enterococcus., Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas sp., Salmonella typhi, S. paratyphi, Shigella sp., Staphylococcus sp. and Streptococcus faecalis and yeasts like Candida tropicalis, Candida glabrata and Candida spp. were detected, is indicative of fecal and water borne contamination of these fruit juices. Multiple Antibiotic Resistance Index Percentage of the pathogens observed to be 17.64-47.05. All the isolates were susceptible to the ten essential oils at 10 µl concentrations. The Minimum inhibitory Concentrations (MICs) of the oils ranged between 0.48-250 µl/ml when studied through tube dilution method. Clove, Azowain, and Mint oils showed better activities against the pathogens in comparison to other oils, with lower MIC values. The essential oils considered in this research showed satisfactory antimicrobial activity and could be used for the development of novel systems for food preservation.

Key words: Antimicrobial activity, Essential oils, Fruit juices, Foodborne pathogens, Food preservation

Introduction

Street foods or fast foods are "ready-to-eat foods and beverages prepared and sold by vendors especially in streets and similar public places" FAO 1989). Street foods such as snacks, fruit & vegetable juices, cold drinks, meals, salads, beverages etc. attract all age groups, especially the younger generations, because of their tastiness, low cost and nutrient value. Street vended fruit juices are well recognized for their nutritive values, mineral, vitamin contents & most important phytochemicals (Sandeep et al. 2001). Contamination of street foods is a major concern and reported worldwide for several epidemics(Mosupye and VonHoly 1999; Mensah et al. 2002, Suneetha et al. 2011; Das et al 2012; Rath and Patra 2012). Food borne illness associated with the consumption of fruit juices at several places have been reported earlier (Chumber et al. 2007; Tambekar et al. 2009).

Different chemical preservatives (benzoic acid, propionic acid, lactic acid, acetic acid, citric acid, sorbic acid, ethylene, sodium diacetate, sodium nitrate, nitrite, caprylic acid, sulphurous acid, sulfite, sulfate, ethyl formate, sodium chloride etc.) are added to food stuffs, so as to inhibit and kill microbes. In addition, certain antibiotics like Auromycin, Streptomycin, Tetramycin are also used as preservatives under chilling condition. However, it is pertinent to discuss here that all the street foods including fruit juices do not require long term preservations because of their short self-life and rapid consumption. Therefore, it is highly essential to inhibit contamination of these street vended foods as well as un- host the pathogen growth. Several natural compounds of plant origin have been reported with antimicrobial properties against food borne pathogens. Further, there is a growing demand for use of natural preservatives in food industries for preservation of food items against costly, harmful chemical/synthetic preservatives. In this regard, essential oils of plant origin could be a good choice, as these oils are edible, with fragrance to human acceptance, antioxidant activities and

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reported to possess antimicrobial properties (Janssen et al. 1986; Kelm et al. 2000, Adeltrudes and Marina 2010). The spice & condiment oils in addition to their antimicrobial activities also increase the quality of street foods by increasing the taste and odour, hence is suggestive of their use in food items including the fruit juices as preservatives.

Keeping this in view, in the present investigation we have made an attempt to study the microbial quality assessment of street vended fruit juices sold in Baripada town, Odisha, India and enhancement of quality of fruit juices by addition of essential oils. The issues addressed in this investigation are: (i) Determination of total aerobic microbial (bacterial & Yeasts) load in the collected fruit juice samples; (ii) to determine the presence of coliforms (if any) in these samples; (iii) study of viability of the pathogens on fruit juices; (iv) evaluation of drug resistance pattern of selected isolates and (v) study of effect of essential oils on the isolates, in order to develop their applications as preservatives in fruit juices.

Materials and Methods

Collection of samples Fruit juices such as Sugarcane, Orange, Mosambi, Grapes were collected from different areas in Baripada town from street vendors in presterilised polythene bags following standard microbiological methods. Data regarding, educational qualification, socio-economic status, sanitation facilities and preparations of samples were collected from the vendors.

pH Measurement The pH of each variety of samples were measured by the help of a pH meter (Systronics-362) in the laboratory. Besides, pH was measured directly by the help of a pH paper at the site of collection.

Coliform test Presence of coliforms in these fruit juices was detected using lactose broth through MPN-3 tube test method (Aneja 1996).

Determination of aerobic bacterial and yeast load by plate count method One ml of sample was subjected to tenfold serial dilution taking 9 ml sterile distilled water, as diluents. From each dilution tube 0.1 ml sample was spreaded over the sterilized, solidified Nutrient Agar (NA), Mac-Conkey Agar(MA), Eosine Methylene Blue Agar (EMB Agar), Urinary Tract Infection Agar (UTI Agar), Sabroud's Dextrose Agar (SDA), Pseudomonas Isolation Agar (PIA), Xylose Lysine Deoxycholate Agar (XLD Agar), Salmonella Shigellal Agar(SS Agar), Thiosulphate Citrate Bile Salt Sucrose Agar(TCBS Agar) for growth and isolation of pathogenic bacteria (all the media were procured from HI-Media , Pvt. Ltd. Mumbai , India and prepared as per manufacturer's instructions). The plates were incubated at 370C for 24 hours. After incubation period viable counts on the plates were recorded. Aerobic bacterial load was determined by calculating the colony forming unit per sample (Colony Forming Units (CFU)/sample = No. of bacterial colonies x dilution factor). Yeasts were enumerated on SDA media.

On the other hand, one loop full of each variety of fruit juices were streaked over different media viz. NA, MA, SS, PA, EMB, XLD, UTI, TCBS, SDA, agar separately. After the incubation period (at 370C for 24 hours), the plates were observed for growth of colonies, selected colonies were isolated from both streak & spread plates.

Identification of the isolates Bacterial isolates were identified based on colony characters, morphology, (gram staining) and through a battery of biochemical tests (Collins and Lyne 1975; Bhat and Myero 1962; Holt et al. 2000). Yeasts were identified based on their growth characters and pigmentation on Hi-chrome Candida Agar (Hi-Media Pvt. Ltd. Mumbai, India).

Study of viability of pathogens in different fruit juices An experiment was designed to study the viability of the pathogens in fruit juices. In this study, E. coli, Bacillus cereus, Enterobacter sp. and Candida glabrata were taken as representative isolates among Gm-ve, & Gm+ve pathogens & yeasts respectively. Two sets of six test tubes were taken, each test tube containing 10 ml of fruit juice for two sets were sterilized. Then 100 µl of freshly grown E. coli, B. cereus, Enterobacter sp. and Candida glabrata culture was inoculated into each test tube separately. One set was incubated at 370C & the other at ambient (Room temperature (30±20C). One loop of sample from each test tube was sub-cultured onto NA plates up to 36 days at an interval of 24 hrs in order to determine the viability of pathogens in the fruit juices.

Antibiotic sensitivity test of the isolates

Selected bacterial and yeast isolates were screened for their antibiogram pattern against 21 antibiotics [Amikacin, (AK, 30 µg); Amoxycillin (Am, 30 µg); Bacitracin (B, 10U); Carbencillin (Cb, 100 µg); Chloramphenicol (C, 30 µg); Ciprofloxacin (Cf, 30 µg); Gatifloxacin (Gf, 30 µg); Gentamicin (G, 10 µg), Methicillin (M, 30 µg); Nalidixic Acid (Na, 30 µg); Nitrofurantoin (Nf, 300 µg); Norfloxacin (Nx, 10 µg); Penicillin-G (P, 10U), Polymyxin-B (Pb, 300U); Tetracycline (T, 10 µg); Triple Sulphas (S3, 300 µg); Vancomycin (Va, 30 µg); Ketokonazole (K, 10 µg); Nystatin-B (Nys-B, 10 µg 0); Fluconazole (Fl, 10 µg); Clotrimazole (Cl, 10 µg)] by disc diffusion method (Bauer et al. 1966). The multiple antibiotic resistance index (%) of a pathogen was determined by using the formula described earlier(Das et al. 2012).

Bioactivity of essential oils against the isolates Ten essential oils [Turmeric rhizome, Turmeric leaf (procured from Oil Technological Research Institute, Jawaharla Nehru Technological university, Anantpur, Andhra Pradesh, India) , black Cumin, Curry leaf, Azowain, Cumin seed (procured from The Southern Spice Products, Madurai, India) Clove, Lemon, Ginger, Mint (procured from Aurosikha, Sri Aurobinda Ashram,Pondichery, India)] were screened for their antimicrobial activities 19 pathogens isolated, by disc diffusion method (Bauer et al. 1966).

Briefly, overnight broth culture of the isolates was swept over Mueller Hinton Agar (MHA) plates by the help of sterile cotton swab as to make a lawn culture of the test pathogen. Sterile filter paper discs (5mm diameter) were placed over the lawn culture at equidistance. Essential oil (10µl) was added over the filter paper discs separately and incubated overnight at 370C. Plates were observed for a zone of inhibition around the discs, indicating susceptibility of the pathogen towards the specific oil. This process was repeated for all the selected isolates & the zone sizes were measured and compared for the degree of antibacterial efficiency of the oils against the test pathogens

Determination of Minimum Inhibitory Concentration of the Essential oils Minimum Inhibitory Concentration(MIC) of the oils, against the isolates was studied by two fold tube dilution method (Das et al. 2012).

Results

In this investigation, 41 samples of four street vended fruit juices such as Sugarcane (n=10), Orange (n=12), Mosambi (n=11), Grapes (n=8) were collected from different localities of Baripada town & was subjected to microbial quality assessment. Information regarding preprocessing, preparation and storage was collected from vendors with total confidentiality is presented in table 1. The pH of the samples observed to be acidic. Highest pH values 3.9-5.6 was observed in case of Sugarcane juice and lowest pH 2.7-3.3 was recorded in case of Grape Juice.

Total aerobic bacterial and yeasts load in fruit juice samples The total aerobic bacterial load of each fruit juice sample found on different media is represented in table 2. On UTI agar media all the fruit juice samples showed more aerobic bacterial load in comparison to other media. However, total aerobic bacterial load and yeasts load ranged between 104- 106 and 102-106 respectively (Table 2). Growth of culturable aerobic bacteria on these media gives a primary indication that these samples are contaminated by selective food borne pathogens.

Confirmation of presence of coliforms The coliform count of each sample was determined by most probable number (MPN 3-tube) method and confirmatory test was done by sub-culturing the same onto EMB agar plates respectively (Aneja 1996). Sixty percent of samples were found to be coliform positive, showed green metallic sheen while one loop of sample from MPN tubes was cultured onto EMB agar plates.

Identification of the Isolates In total 153 bacteria were isolated from 41 different fruit juice samples based on their growth and colony morphology on different media. Out of which, 129 (84.31%) isolates were identified by studying their colony characterstics, morphology & biochemical reactions whereas, 24 (15.68%) isolates remained unidentified. The identified isolates were assigned to 11 different genera (Arizona sp., Bacillus sp., Escherichia coli, Enterobacter sp., Enterococcus., Klebsiella pneumonia, Proteus mirabilis, Pseudomonas sp., Salmonella typhi, S. paratyphi, Shigella sp., Staphylococcus sp. and Streptococcus faecalis and yeasts like Candida tropicalis, Candida glabrata and Candida spp.) & the details are presented in Table 3.

During our investigation Klebsiella pneumoniae observed to be the dominant species followed by E. coli and Bacillus sp. and yeast (Table 3). Least dominance was observed in case of Salmonella paratyphi & Shigella dysenteriae. Both E. coli & yeasts were isolated from 70% of the fruit juices studied. Whereas, K. pneumoniae, Enterobacter sp., Pseudomonas sp. were recovered from (60%) of the samples. In contrast, Bacillus sp. were reported from 80% of the fruit juices studied.

Bacillus sp. (n=18) were subcultured on Hi-chrome Bacillus agar media & were identified based on their growth characters & pigmentation. 38.88% of the isolates were identified as Bacillus subtilis, whereas, B. cereus, B. megaterium, B. coagulans & B. thuringiensis ranged up to 22.22%, 16.66%, 11.11% & 11.11% respectively. Yeasts (n=17) were identified by growing on Hi-chrome Candida agar, 29.41% (n=5) and 41.17% (n=7) of the isolates were identified as Candida glabrata and Candida tropicalis respectively. Whereas, 5 (29.41%) of the yeast isolates could not be identified based on their growth and pigmentation on Candida Agar (Hi-Media Pvt. Ltd. Mumbai, India)

Viability of the pathogens in fruit juices An experiment was designed to study the viability of pathogens in these fruit juices, by inoculating E. coli, B. cereus & Enterobacter sp. and Candida glabrata as representative isolated pathogens, into various fruit juices and incubating at both room temperature & at 370C up to 36 days. It was observed that E. coli, B. cereus, Enterobacter sp. and C. glabrata were viable up to 36 days at room temperature in these fruit juices but, were viable up to 29 days at 370C.

Antibiogram pattern of the isolates The antibiotic sensitivity pattern of the isolates were studied by disc diffusion method and the multiple antibiotic resistance (MAR) index, was determined [23]. From the antibiogram studies it was observed that all the isolates were resistant to Penicillin & Methicillin (Table 4). The antibiotics to which all isolates were commonly sensitive were Ciprofloxacin, Norfloxacin, Amoxycillin, Gentamycin, Tetracycline, Amikacin, Chloramphenicol, Gatifloxacin, Nalidixic acid, Nitrofurantoin, Polymixin-B. The multiple antibiotic resistance index varied from 17.64 – 47.05%. Shigella dysenteriae showed highest MAR index (47.05%) followed by Arizona (41.17%), Klebsiella (35.29%), Enterobacter, Pseudomonas, Salmonella, Proteus, Enterococcus spp. showed similar MAR index (29.41%). Candida glabrata and Candida tropicalis were resistant to Fluconazole and were susceptible to Ketoconazole, Clotrimazole and Nystatin B, represented a MAR index percentage 33.33.

Susceptibility of the Pathogens to Essential oils
Susceptibility of the pathogens towards different essential oils is presented in table 5. It was noted that, all the pathogens were susceptible to clove oil, black cumin, curry leaf, azowain, cumin seed, lemon, mint essential oils at 10 µl/disc, however, a degree of variability was reported in the action of essential oils in terms of zones of inhibition. Staphylococcus aureus, Salmonella paratyphi, B. coagulans, B. subtilis, B. megaterium, B. thuringiensis, Candida tropicalis showed highest percentage of susceptibility i. e 100%, towards the EOs used, whereas, Enterobacter & Proteus showed lowest degree of susceptibility i.e. 40% against the oils studied.

Minimum Inhibitory Concentrations (MICs) of the oils
The minimum inhibitory concentration of the oils ranged between 0.48-250ul/ml (Table 6). All the oils showed lower MIC values against the Candida spp. whereas, Turmeric rhizome, turmeric leaf and ginger oil showed least antibacterial activities with higher MIC value of 3.9ul/ml against these pathogens. The oils showed a lower MIC range of 0.48-1.95ul/ml against Bacillus megaterium except lemon oil. The MIC values of the oils corroborates with our earlier findings during this investigation i. e. oils with higher zones of inhibition represented lower MIC values.

Discussion

Fruit juices could be contaminated through the poor quality of water used in washing, improper handling, use of unsterilized storage container & unhygienic practices. The microorganisms normally present on the surface of raw fruits & vegetables may consist chance of contaminations from the soil or dust. Contamination of street vended fruit juices especially Sugarcane, Orange, Mosambi and Grapes which were studied in this investigation, are increased by unhygienic processing. While comparing the bacterial load socio-economic status of the vendors it was observed that many of vendors were poorly educated, unlicensed, untrained in food hygiene with little or no knowledge about the causes of food borne diseases. Vendors usually prepare and serve the fruit juices in bare and unwashed hands which is one of the sources of contamination (Table 1). During the investigation, we observed that, the storage stands were not properly cleaned or sterilized and also storage of food in refrigerator is usually unavailable. Mixing of ice in fruit juices is a common practice by the vendors. Manufacture of ice from contaminated water and unhygienic preservation could further attribute to fruit juice contamination. Unhygienic fruit handling in the poorly sanitized environmental conditions under which vendors operate the juices become contaminated with harmful bacteria (Mukhopadhaya et al. 2011), which is in proper agreement to our findings. The pH of the studied fruit juices reported to be acidic. Several pathogenic bacteria reported to be grown in food samples at acidic pH range of 2.7-5.6 (Silva et al. 1999; Chang and Kang 2004).

Ahmed et al. (2009) revealed that the freshly prepared fruit juice contain various microorganisms such as E. coli, B. cereus, Staphylococcus aureus, Salmonella sp., Streptococcus sp. etc., as reported in this investigation. The bacterial contamination of fruit juices with E. coli, Salmonella sp., Proteus sp., Enterobacter aerogens, Klebsiella pneumoniae & Pseudomonas aeruginosa & other coliform bacteria is generally an indication of fecal contamination of the water often used by vendors for washing their utensils & hands before cutting the fruits (Chukwu et al. 2010, Bagde and Tumane 2011)]. A study on the quality of sugarcane juice showed high occurance of thermotolerant coliforms that in agreement with our investigation(Olivieira 2006). Presence of coliforms was reported in 60% of the samples, indicating a high risk, that other pathogenic organisms might have also contaminated the fruit juices.

Occurrence of Bacillus species in these fruit juices implicated the ubiquitous nature of bacterial spores especially in dusty road side locations. In general, the presence of mesophilic spore formers Bacillus cereus in food is of great significance since this organism produce heat sensitive and heat stable toxins associated with food poisoning (Walls and Chuyate 1998). Detection of B. thuringiensis, in these fruit juices again is indicative of soil contamination of these samples, through improper handling. Spoilage of food could be attributable to contamination with aerobic acid-tolerant bacteria as well as yeasts & molds. Presence of bacterial species like Staphylococcus and yeasts (Candida glabrata, C. tropicalis) is indicative of cutaneous infections and their of transfer to these fruit juices due to mishandling (Suaad and Eman (2008).

Pathogens like E. coli, B. cereus & Enterobacter sp. and Candida glabrata were viable in these fruit juices up to 36 days and 29 days when incubated/stored at room temperature (25±20C) and at 370C respectively. The viability of these organisms in these samples for a long period is due to highly tolerance of wide range of temperature, pH and nutrition value of these fruit juices. Viability of these pathogens in street vended food items is well recorded in literature [8]. Earlier studies have demonstrated the survival of microorganisms, including human pathogens in various juices. E. coli remained viable in apple and orange juices up to 24 days. Viability of Vibrio cholerae, Bacillus sp. and E. coli in ragi (Eleusine coracona) gruel even at ambient temperature and at 370C up to 30 days have been reported in literature (Subramanyam and Rath 1994). Survival of these pathogens in these fruit juices pose a hazard, as sometimes the left out juices are stored for a longer period at room temperature by the vendors.

From the antibiogram studies it was observed that the Multiple Antibiotic Resistance Index percentage ranged between 17.64-47.05. In agrrement to our findings, Das et

al. (2012) also reported higher MAR index of food borne bacteria from a popular Indian street food Panipuri. Similarly, Rath and Patra (2012)], also observed a higher MAR % index against these pathogens isolated from street vended foods such as Panipuri, Dahibara and Chaat. Development of multiple antibiotic resistance among pathogens in a clinical study is of global concern today. Presence of multi drug resistant pathogens in food samples such as fruit juices as observed in this study is also alarming. Considering the development of multiple antibiotic resistant microorganisms, the scientific communities in the recent past are relying on herbal medications all over the world. While studying the antimicrobial activity of the essential oils, it was observed that pathogens like Streptococcus faecalis, Staphylococcus sp., Salmonella paratyphi, Candida tropicalis, Bacillus thuriengenesis, B. subtilis, B. megaterium, B. cereus were susceptible to all the ten essential oils at 10 µl concentrations per disc. In cases, the zones of inhibition are well compared to standard antibiotics used (Table 4 & 5). In specific cases we observed smaller zones of inhibition which, could be attributable to the crudeness of the components present in the oil and/or the antagonistic activities of the oil components present. Antagonistic activity of mixture of essential oils is reported against Pseudomonas aeruginosa, Bacillus cereus, Salmonella typhimurium, Shigella boyedii (Rath et al. 2002). Behera and Rath (2011) also reported the antagonistic activity of leaf essential oil mixtures of Turmeric (Curcuma longa L.) against Shigella spp. The Minimum Inhibitory Concentration of the essential oils against the test pathogens ranged between 0.48-250 µl/ml. Clove, Azowain, and Mint oils showed better activities against the pathogens with lower MIC values. Lower MIC values of essential oils (lemon, ginger, mint & turmeric oil) against pathogenic bacteria including E. coli, B. cereus and members of Enterobacteriaceae is being reported in literature (Das et al 2012; Rath and Patra 2012) as observed in this investigation. The activities of the oils reported to be bactericidal and fungicidal in nature, as no growth was observed on nutirient media, when a loop full of the sample was cultured from the MIC dilution tubes. Antibacterial activity of Ocimum, Ginger, Turmeric, Japaneese mint essential oils against the above food borne pathogens is also reported (Das et al 2009; Gupta et al. 2004). Rath et al. (2005) observed the antibacterial activity of Lime and Juniper essential oils against methicillin resistant Staphylococcus aureus (MRSA). Burt (2004) reviewed the antibacterial properties and potential application of essential oils in foods .

During the investigation a varying degree of activity of the essential oils was observed against the test pathogens may be due to the intrinsic tolerance of microorganisms and the nature of combination of compounds present in the essential oils. Many authors have reported the better antimicrobial activity of essential oils against Gram-negative bacteria (Friedman et al. 2002; Burt 2004; Rath 2007; Rath et al 2012). But in contrast to these observations, in this investigation we observed better antibacterial activity (with lower MIC) of all the essential oils against Bacillus megaterium a Gram-positive isolate. Lower MIC values of Clove, Black Cumin, Curry leaf, Azowain, and Cumin seed essential oils are also, reported against Candida spp. Further, it is to add that the essential oils are rich in terpenes (monoteroenes, oxygenated monoterpenes and sesquiterpenes). However, the mode of action of terpenic constituents (essential oils) on microorganisms is not fully understood. But, in view of their hydrophobicity, it is considered that they are involved in mechanisms such as cytoplasmic membrane, coagulation of cell contents and disruption of the proton motive force (Burt 2004). Senhaji et al.(2007) observed the antibacterial activity of Cinnamon essential oil against E. coli O157: H7 by outer membrane disintegration and increasing the permeability of ATP through cytoplasmic membrane indicating that physical contact of essential oil components to the microbial surface is necessary for execution of their antimicrobial properties. Hydrophobicity of essential oils and their components that enables them to make partitions in the membrane, rendering permeability and leading to leakage of cell contents resulting in death of microbial cells. Significance of essential oils has incredibly increased, due to the antimicrobial activities to control food pathogens and food native microflora and the knowledge of possible mechanism of these oils (Singh et al. 2002; Sabulal et al. 2006). Uses of essential oils as natural food preservatives is being discussed in literature too (Rath 2007)].

Conclusion

In this investigation we have reported presence of pathogenic bacterial and yeast strains in street vended fruit juices like Sugarcane, Orange, Mosambi and Grapes. This could be attributable to, unhygienic handling, use of low grade fruits or raw materials, improper storage, use of ice (prepared with contaminated or unsterilized water) as coolants etc. We also observed, the antimicrobial potential of ten essential oils against these pathogens. Moreover, in this study the effect of some essential oils against spoilage microorganisms was ascertained at very low concentrations, most importantly, these oils do not alter the taste and odour of food items at low concentration, suggesting their use as novel systems of food preservation. However, further research is necessary to investigate the possible interaction between oils and food components, before their use as natural preservatives.

Table 1: Data representing status of the vendors with respect to sample preparation

Sl No.	Type of fruit juice	Collection sites	Type of Vendor Roadside/Restaurant	Educational standard	Socio-economic Status	Cleaning of juicer 1/2/3/4	Peeling of fruits Y/N	Peeling of fruits by hand/machine	After peeling cutting is done Y/N	Fruits used washed prior to peeling Y/N	Does the fruits are soaked before juicing after peeling Y/N	How the fruits are stored prior to use	Is ice added Y/N
1	Sugarcane(03)	Kochari	Roadside	V	Poor	4	Y	Hand	N	N	Y	Gunny bags	Y
2	Sugarcane(03)	Balasore Golei	Roadside	IX	Poor	3	Y	Hand	N	Y	N	Gunny bags	Y
3	Sugarcane(04)	Station Bazar	Roadside	VIII	Poor	4	Y	Hand	N	N	Y	Gunny bags	Y
4	Orange (04)	Kochari	Roadside	IX	Medium	4	Y	Hand	N	N	N	Basket	Y
5	Orange (04)	Hot and Cold bar	Restaurant	X	Medium	Many times	Y	Hand	N	N	Y	Basket	Y
6	Orange (04)	Balasore Golei	Roadside	IX	Poor	4	Y	Hand	N	N	Y	Basket	Y
7	Mosambi (05)	Balasore Golei	Roadside	IX	Poor	4	Y	Hand	N	N	Y	Basket	Y
8	Mosambi (06)	Kochari	Roadside	IX	Medium	4	Y	Hand	N	N	N	Basket	Y
9	Grapes (04)	Kochari	Roadside	IX	Poor	4	N	N	N	Y	Y	Basket	Y
10	Grapes (04)	Balasore Golei	Roadside	IX	Poor	4	N	N	N	Y	Y	Basket	Y

1- Once daily; 2- After each preparation; 3- Only once at the end of the day; 4- 2/3 times at different time intervals, Y-yes; N-No

Table 2. Total aerobic bacterial load of samples*

Sample No	Medium									
	NA	MA	UTI	XLD	SS	TCBS	EMB	SDA	PDA	PA
Sugarcane juice (n=10)	$9.4x10^5$	$2.7x10^5$	$2.6x10^6$	$1.7x10^5$	$2.1x10^5$	$1.3x10^4$	$2.3x10^5$	$2.1x10^5$	$2.3x10^6$	-
Orange juice (n=12)	$1.1x10^6$	$1.4x10^5$	$1.3x10^6$	$6.x10^4$	$1.2x10^5$	$4.9x10^5$	$1.0x10^6$	$1.4x10^2$	$1.5x10^3$	-

Mosambi juice (n=11)	$2.6x10^6$	$1.6x10^3$	$2.9x10^6$	$5.3x10^5$	-	-	-	-	-	$2.6x10^6$
Grapes juice (n=08)	$3.8x10^5$	$3.0x10^4$	$1.4x10^6$	$3.6x10^5$	$3.8x10^5$	-	$1.1x10^6$	$1.5x10^6$	$1.4x10^6$	$1.5x10^5$

*Bacterial load was determined through spread plate method.

- No valid count. All the dilutions represented>300 CFU, as a result no valid count could be recorded.

NA- Nutrient Agar; MA- MacConkey Agar; UTI-Urinary Tract Infection Agar; SS-Salmonella-Shigella Agar; TCBS-Thiosulphate Citrate Bilesalt Sucrose Agar; EMB-Eosine Methelene Blue Agar; SDA-Sabouraud's Dextrose Agar; PDA- Potato Dextrose Agar; PA- Pseudomonas Agar.

Table 3: Predominance of isolates

Isolates	**Total number of pathogen isolated(n)**	**Percentage dominance (%)**
Arizona sp.	3	2.32
Bacillus sp.	18	13.95
E. coli	18	13.95
Enterobacter sp.	11	8.52
Enterococcus sp.	3	2.32
Klebsiella pneumoniae	20	15.50
Proteus mirabilis	5	3.87
Pseudomonas sp.	9	6.97
S. paratyphi	1	0.77
Salmonella typhi	10	7.75
Shigella sp.	1	0.77
Staphylococcus sp.	9	6.97
Streptococcus faecalis	4	3.10
Yeast	17	13.17
Total	**129**	**100**

The % Values represent of the identified isolates (n=129) only; Unidentified 24(15.68%). Total no. of isolates 153.

Table 4: Antibiotic sensitivity pattern of the isolates

Isolates	Antibiogram		
	Sensitive to	**Resistant to**	**MAR%**
Arizona sp.	Am (18), Ak (17), C(22), Cip (24), Gf (7), Na (17), Nx(25), Nf(26), Pb(6), T(14), Cb(19)	B,Gen,M,P,S3,Va	35.29
Bacillus cereus	C(29), Gf (30), Cip(28), B(18), Ak, (21), Am(32), G (20), Pb (10), Va(18), Nf(30), Cb(15), T(30), S3 (16), Nx(14)	Na, P,M	17.64
E. coli	S3 (24), Nx (28), Am(25), B(11), Va (12), Pb (12), C(22), G (17), Na (24), Gf (26), Ak(18), T(15), Nf (16), Cip (30)	P,M,Cb	17.64
Enterobacter sp.	NX (26), S3 (22), Na (22), T (18), Am (9) Nf (14), Ak(19), Pb(11), C(24), Cip (32), Gf(25), G(18).	P,Cb,B,Va,M	29.41
Enterococcus sp.	Am(27), Ak(20), C(27), Cip (35), Cb(25), Gen (20), Gf(33), Na(21), Nx(10), Nf(22), Pb(19), T(18.)	B,M,P,S3,Va	29.41
Klebsiella pneumoniae	Am(20), Ak(18), C(22), Cb(17), Cip (30), Gen(19), Gf(24), Na (18), Nx(10), Nf(12), Pb(18)	B,M,P,T,S3,Va	35.29
Proteus sp.	Am (18), Ak (17), C (25), Cb(13), Cip (26), Gen(19), Gf(17), Na (17), Nx(24), Nf(16), Pb (10), T(10)	B,M,P,S3,Va	29.41
Pseudomonas sp.	T (18), C(22), Pb(10), GF(26), AM (10), S3 (19), G(17), CIP (25), AK(21), NF (17), NX (22), Na (22)	Va, B, M,P, Cb	29.41
Salmonella sp.	Cip (30), Gf (25), Ak(20), Na (24), C (22), Pb (10), Am(18), Nx (30), G(20), T(17), S3 (15), Nf(20)	M,Va, B, Cb, P	29.41
Shigella dysenteriae	Cip (45), Ak(26), Gf(40), Nx(37), Pb (14), G (25), Am(28), T(24), S3(20)	M, Na, Nf, B, Va, C,P, Cb	47.05
Staphylococcus sp.	Nx (27), S3,(21), G(25), Nf (23), Cip(36), B(13), Ak (25), Pb(11), Gf(37), Am (38), Va (15), C(24), T(26).	Cb, P, Na, M	23.52
Streptococcus faecalis	C (19), Cip (35), Va (16), Nx (20), S3 (16), Ak (20), Gf (34), Am (10), Na(17), G (17), T(22), Nf (18), B(10)	Cb, Pb, P, M	23.52
Candida glabrata	K(10), Nys-B(18), Cl(12)	Fl	33.33
Candida tropicalis	K(13), Nys-B(17), Cl(12)	Fl	33.33

MAR- Multiple Antibiotic Resistance Index; values in parentheses represent zone of sensitivity in mm.

Table 5: Susceptibility of isolates towards different essential oils.*

Pathogens	Essential oils (Zone of inhibition in mm)										
	Turmeric rhizome	Clove	Black cumin	Curry leaf	Azowain	Cumin seed	Lemon	Turmeric leaf	Ginger	Mint	Susceptibility (%)
Arizona sp.	11±0.0	24.5±0.7	11±0.0	NZ	25±0.0	12.5±0.7	11±0.0	NZ	NZ	15.5±0.7	70
B.cereus	12±0.0	30±0.0	30±0.0	30±0.0	30±0.0	NZ	NZ	11.5±0.7	11±0.0	12±0.0	80
B.coagulans	16±1.4	21±1.4	13.5±0.7	12±1.4	39±1.4	35.5±0.7	14±0.0	15±0.0	11±0.0	30±0.0	100
B.megaterium	17±4.2	17±1.4	51±1.4	17±1.4	50±0.0	23.5±0.7	17.5±0.7	18±0.0	24.5±0.7	45.5±0.7	100
B.subtilis	11±0.0	16±2.8	11±0.0	11±0.0	22.5±0.7	12±0.0	11±0.0	11.5±0.7	11±0.0	11±0.0	100
B.thuringiensis	11±0.0	24.5±0.7	11.5±0.7	11±0.0	21±1.4	12±0.0	11.5±0.7	13±1.4	11±0.0	12.5±0.7	100
Candida glabrata	11±0.0	33±1.4	18.5±0.7	13.5±0.7	37±4.2	30±0.0	16. 5±2.1	15±0.0	12±0.0	28.5±0.7	90
Candida sp.	13±0.0	28±2.8	11.5±0.7	11.5±0.7	31±1.4	20±0.0	11±0.0	12.5±0.7	11±0.0	12.5±0.7	90
Candida tropicalis	11±0.0	32±0.0	28±0.0	18±0.0	30±0.0	30±0.0	30±0.0	13.5±0.7	12±1.4	30 ±0.0	100
E.coli	11±0.0	21.5±2.1	11±0.0	11±0.0	24±1.4	NZ	NZ	11±0.0	11±0.0	12±0.0	80
Enterobacter sp.	NZ	30±0.0	NZ	NZ	12.5±0.7	12.5±0.7	NZ	NZ	NZ	12±1.4	40
Enterococcus sp.	11±0.0	28±2.8	11.5±0.7	11±0.0	11±0.0	11±0.0	11±0.0	11±0.0	11±0.0	NZ	90
Klebsiella pneumoniae	NZ	24.5±0.7	11.5±0.7	11±0.0	18±0.0	12.5±0.7	12±0.0	13±0.0	NZ	14.5±0.7	80
Proteus mirabils	NZ	26.5±2.1	11±0.0	11±0.0	11±0.0	NZ	NZ	NZ	NZ	NZ	40
Pseudomonas sp.	NZ	19±1.4	NZ	11±0.0	25±0.0	13±1.4	12±0.0	12±0.0	NZ	13±0.0	70
Salmonella paratyphi	11±0.0	11±0.0	12±0.0	11±0.0	11±0.0	11±0.0	11±0.0	11±0.0	11±0.0	11±0.0	100
Salmonella typhi	NZ	21± 1.4	11±0.0	11±0.0	24±2.8	12±0.0	11±0.0	11.5±0.7	11±0.0	12.5±0.7	90
Shigella sp.	11±0.0	11±0.0	11±0.0	11±0.0	NZ	11±0.0	11±0.0	11±0.0	11±0.0	11.5±0.7	90
Staphylococcus sp.	11.5±0.7	23.5±2.1	11±0.0	12±1.4	25.5±2.1	11.5±0.7	11.5±0.7	12±1.4	11±0.0	21.5±4.9	100
Streptococcus faecalis	11±0.0	22.5±0.7	11±0.0	11±0.0	11.5±0.7	11±0.0	11±0.0	11±0.0	11±0.0	11±0.0	100

*Oil was loaded 10µl/disc. NZ-No zone of inhibition, values represent zones of sensitivity in mm.

Table 6: Minimum Inhibitory Concentration (MIC) of essential oils.*

Pathogens	MIC of Essential oils (µl/ml)									
	Turmeric rhizome	**Clove**	**Black cumin**	**Curry leaf**	**Azowain**	**Cumin seed**	**Lemon**	**Turmeric leaf**	**Ginger**	**Mint**
Arizona sp.	3.9	1.95	3.9	250	1.95	3.9	3.9	250	250	1.95
B.cereus	3.9	0.97	0.97	0.97	0.97	250	250	250	3.9	3.9
B.coagulans	1.95	3.9	3.9	3.9	0.97	0.97	3.9	3.9	3.9	0.97
B.megaterium	1.95	1.95	0.48	1.95	0.48	1.95	3.9	1.95	1.95	0.48
B.subtilis	3.9	1.95	3.9	3.9	1.95	3.9	1.95	3.9	3.9	3.9
B.thuringiensis	3.9	0.97	3.9	3.9	1.95	3.9	3.9	3.9	3.9	3.9
Candida glabrata	3.9	0.97	1.95	3.9	0.97	0.97	1.95	1.95	3.9	1.95
Candida sp.	3.9	1.95	1.95	3.9	0.97	1.95	3.9	3.9	3.9	3.9
Candida tropicalis	3.9	0.97	1.95	1.95	0.97	0.97	0.97	3.9	3.9	0.97
E.coli	3.9	3.9	3.9	3.9	1.95	250	250	3.9	3.9	3.9
Enterobacter sp.	250	0.97	250	250	3.9	3.9	250	250	250	3.9
Enterococcus sp.	3.9	1.95	3.9	3.9	3.9	3.9	3.9	3.9	3.9	250
Klebsiella pneumoniae	250	1.95	3.9	3.9	1.95	3.9	3.9	3.9	250	3.9
Proteus mirabils	250	1.95	3.9	3.9	3.9	250	250	250	250	250
Pseudomonas sp.	250	1.95	250	3.9	1.95	3.9	3.9	3.9	250	3.9
Salmonella paratyphi	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9
Salmonella typhi	250	1.95	3.9	3.9	1.95	3.9	3.9	3.9	3.9	3.9
Shigella sp.	3.9	3.9	3.9	3.9	250	3.9	3.9	3.9	3.9	3.9
Staphylococcus sp.	3.9	1.95	3.9	3.9	1.95	3.9	3.9	3.9	3.9	1.95
Streptococcus faecalis	3.9	1.95	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9

*****Minimum Inhibitory Concentration (MIC) of the oil was determined through twofold tube dilution method. The Media was supplemented with 0.5% of tween 20 to facilitate miscibility of the oil in the

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