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An Analytical Approach on Enzymatic Degradation of Biochemical Components by Isolated Food Contaminant Pathogens from Sugarcane Juice of Street Salesman

A.P. Chowdhury, A. Das[1], R.R. Singh[2], H. Goswami[2], M.P. Bhuyan[3]

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ABSTRACT

Background: Sugarcane is an important agro-industrial crop as a worldwide manufacturing plant. Scientifically it is the so-called *Saccharum officinarum*. Sugarcane juice is dark brown golden in color with medium flavor intensity contains caramel, butterscotch and green flavor and sucrose hydrolyzed to an equimolar mixture of glucose and fructose providing instant energy transported by fluid thermodynamics. The expected research based on a new implementation of harmful pathogens against roadside sellers of sugarcane juices. The work is currently reviewed and renewed for automated analysis techniques with verified street juice samples at Guwahati city in Assam. Following spring to summer seasons, the favorite juice to people of all ages in India is supposed to health-threatening for public health concerns. Respective doctors and Practitioner carry out their treatment against this pathogen along with antibiotic supplements unknowingly the etiological source about UTI or GI tract infections.

Methods: Here researchers finding (Chowdhury *et al.,* 2019) are to analyze and categorize the biochemical fermentation activities of pathogens with significant enzyme degrading analyzed from sugarcane juice against gut acting probiotics, responsible for food toxicity and intestinal disorder. Diagnostic microbial activities in well diffusion studies are followed by automated analysis for significant provoking of health status.

Result: Epidemiological studies were concerned to identify risk factors through the comparison of incidence and prevalence between groups exposed and not exposed to risk factors. Probabilities of disease occurrence can be compared using measures of strength of association or potential impact. The studies are compared with the graph and its data implementations to find out the significant level for beneficial sources.

Key words: Automated analysis, Biochemical degradations, McFarland scale, Test organisms, Well diffusion.

INTRODUCTION

Sugarcane is a large perennial tropical grass belonging to the Tribe Andropogene of the Family Gramineae (Aparna 2011; Balish and Wanger 1998). The present study was carried out to discuss the bacteriological profile of sugarcane juice sold at the roadside (Abdallah 2016; Collins 1999; Diviya and Baskaran 2020). Quantification of the target analysts was using by 40 juice samples respectively for the detection of harmful contaminants.

Epidemiology is the study of out breaking sites (Endemic, Epidemic and Pandemic) of an infectious pathogen, its infection rate (virulence) on about a population (Sporadic) and establishment of measuring steps (Prevention and Control) against it (Bengmark 1998; Ogura and Chen 2001). Only a few infectious bacterial and fungus particles are needed to cause an infection that can exist in sugarcane juice as contaminants (Edlund 2010). This amount can readily be acquired through contact with contaminated hands and objects (Water, ice cubes, Dry sugar cane, mechanical apparatus).

Demography of sales corner in Guwahati city

Most of the villagers in Assam carry their serials and sugarcane by boat across the river Brahmaputra later these are transported by pickup van from different cultivated places. The sells men buy this sugarcane as a bundle from different wholesale of markets in Gauhati city. Then the salesman along with his slicing machine (manual or generator operated) to slice and mash the sugarcane for preparing juice by extracted residues of sugarcane mixing with an ice cube on the street sides or unhygienic places of the footpath (Gaskins 1999; Vaidya 2008).

Department of Botany, Gauhati University, Guwahati-781 014, Assam, India.
[1]M/S Hydrocraft Industry, Guwahati-781 006, Assam, India.
[2]Assam Downtown University, Panikhaiti-781 068, Assam, India.
[3]SRL Diagnostic, Bhangagarh, Guwahati-781 005, Assam, India.

Corresponding Author: A.P. Chowdhury, Department of Botany, Gauhati University, Guwahati-781 014, Assam, India. Email: avranu132@gmail.com

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Exposure assessment

Sucrose of sugarcane leads the growth of airborne microbes on the surface in unhygienic condition → Airborne contaminants of sugarcane parenchyma interacts with its pathogenic constituents from biochemical nutritional prophylaxis → Waterborne microbes from ice cubes propagated very faster rather than others while mixing with sucrose during juice preparation (Schultz and Baldour 2000; Sarella 2002; Vanderhoof 2000).

Objectives

Considering all the aspects discussed previously, the present study was undertaken to perform the following objects:

1. To determine and compare the hygienic quality of the collected samples.
2. To identify the causative organism or the microbial viability are responsible for the spoilage of sugarcane juice.
3. Isolation and purification of contaminants from samples to signify for public health assurance.

MATERIALS AND METHODS

Sampling sites and number of sampling

Ten sampling sites were visited in the metropolitan area of Gauhati to collect thirty samples. Samples were collected from 15th July 2018. *In vitro* analysis for isolation and identification were screened accomplished within six month at the microbiology laboratory in SRL Diagnostic, Assam, India.

Morphological and physicochemical characteristics of the isolate

Isolates were characterized by considering the colony morphology. The effective isolate was grown on different agar media and 0.5% yeast extract agar medium. After 10 days at 20-22°C, under aerobic conditions, cell shape and colony appearance of selected organisms were examined by phase-contrast microscope to determine the cell morphology.

Cell morphology in liquid culture: Isolates were grown in GY medium containing 10% glucose at 30°C and pH 5.00. After 1 day, culture was diluted and placed under a phase-contrast microscope.

Optimum Temperature for growth of selected isolates was allowed to grow in 250 ml flask containing 50 ml GY medium containing 10% glucose with initial pH 5.0 at a different temperature such as 20°C, 25°C, 30°C, 34°C and 37°C. Growths were measured by diluting culture and keeping the 496 to 500 nm.

Optimum pH for growth of selected isolates was allowed to grow in 250 ml flask containing 50 ml GY medium containing 10% glucose at 30°C with different initial pH such as 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0. Growths were measured by diluting culture and keeping the absorbance 496 to 500 nm.

Biochemical characteristics are more important than morphological characteristics in the identification of microorganisms. For this reason, the following studies will guide to be made on the physiological activities of the selected microbes. Catalase Reaction (Claus, 1995) is capable of decomposing hydrogen peroxide into water and molecular oxygen. The ability to utilize citrate as a source of carbon and energy can be used to distinguish certain grain negative rods. For this test, citrate medium can be inoculated with a very small amount of 48 to 72 hours old culture and incubated at 35°C for 48 hours. The appearance of turbidity or growth in the medium will be indicated the utilization of citrate. Deep Glucose Agar Test (Khan, 1962) was used to be classified with relation to free oxygen as strict aerobes, facultative anaerobes, micro-aerophilic and strict anaerobes. Fermentation Test (Smith, 2003) was of considerable significance in the identification and classification of bacteria. The microorganisms differ in their ability to ferment different carbohydrates. Some of them upon fermentation of carbohydrates produce both acid and gas, some produce only acid, no gas, some produce only alkali and still others that cannot ferment carbohydrates at all. Gelatin Liquefaction (Collins and Lyne, 1984) helped to identify microorganisms that possessed the enzyme gelatinase, which was capable of hydrolyzing gelatin. This test indicates the presence or absence of the enzyme gelatinase in the tested organism. Hydrolysis of Starch (Collins and Lyne, 1984) revealed the presence of the enzyme amylase in the organisms. Proteolysis Test (Smith, 2003) was used to be indicated the presence or absence of the enzyme proteinase in the organisms. This enzyme was capable of hydrolysis of protein.

Implements of cultured isolates in VITEK® 2 Compact system (Version: 08.01)

The culture of one loopful sample from MacConkey agar plates was used to transfer in The VITEK® 2 Compact system at SRL Diagnostic, Assam. The innovative VITEK 2 technology includes an expanded identification of Bacterial colonies in laboratory testing. After 24 hrs incubation of colonial growth from pure culture, colonies were needed for fast and accurate microbial identification by an implemented database, for accurate results depending on the rapid outcomes with minimal training time.

Estimation of enzymes produced by micro-organisms (Chettri and Tamang, 2014)

Another important reason for sugarcane extracted juice into producing enzymes that also provide very useful services (Mann and Singh 1996; Rao 1984). During food fermentation microorganisms produce enzymes to break down complex compounds to simple bio-molecules for several biological activities such as proteinase, amylase, mannanase, cellulase and catalase. So the estimation of degrading enzymes for long storage of sugarcane juice was applied.

RESULTS AND DISCUSSION

The main component of Sugarcane juice is sucrose or saccharose, which is a disaccharide composed of a molecule of glucose connected *via* an [α (1-2)] glycosidic bond to a molecule of fructose. After collections, samples were assembled for 10 fold dilutions and screened out to observe colony counting and reading out optical density.

Colony counting (McFarland *et al.*)

Clinical data confirming the effectiveness of probiotics in preventing tentative diagnosis (TD) is relatively limited. Nevertheless, prophylaxis of TD constitutes one of the largest markets for probiotics in Europe. McFarland *et al.* performed a meta-analysis of studies and found that isolates significantly prevented TD (RR ¼ 0.85, 0.001). No practice guidelines for the use of enzymatic degradation rate of estimated bacterial propagation in sugarcane juice as prophylaxis of TD are currently available (Chowdhury *et al.* 2019).

Biochemical studies of the selected isolates

The physiological characters of all the isolates *i.e.* their growth at a different temperature, pH, their salt (NaCl) tolerance and heat resistance were studied following the procedures described in Table 2.

Table 1: Screening of collected samples in an acceptable range based on optical density.

Dilution samples	Optical density (496-500 nm)	Colony counts in numbers	Range of acceptable unit	
			Range (OD) 0.20 -0.85	Range (mcf) 0-1.8
GS1	1.70	4.02	Non-acceptable	
GS2	0.26	3.24	Non-acceptable	
GS3	0.56	2.98	Non-Acceptable	
MS4	0.50	2.44	Non-acceptable	
MS5	0.80	4.06	Non-acceptable	
MS6	0.30	1.08	Acceptable	
JS7	2.68	5.0	Non-acceptable	
JS8	0.38	2.6	Non-acceptable	
JS9	0.044	2.0	Non-acceptable	
GMS10	0.12	3.6	Non-acceptable	
GMS11	0.22	4.3	Non-acceptable	
GMS12	0.14	5.6	Non-acceptable	
DS13	1.8	4.8	Non-acceptable	
DS14	1.25	5.3	Non-acceptable	
DS15	0.40	1.4	Acceptable	
US16	0.11	4.96	Non-acceptable	
US17	0.17	1.02	Non-acceptable	
US18	0.12	2.6	Non-acceptable	
PS19	1.79	3.88	Non-acceptable	
PS20	1.37	6.1	Non-acceptable	
PS 21	2.16	4.55	Non-acceptable	
FS 22	1.92	6.4	Non-acceptable	
FS23	0.61	4.2	Non-acceptable	
FS24	0.61	1.12	Acceptable	
KS25	1.11	2.4	Non-acceptable	
KS26	1.56	2.7	Non-acceptable	
KS27	0.70	1.03	Acceptable	
SS28	2.92	4.7	Non-acceptable	
SS29	1.1	3.23	Non-acceptable	
SS30	1.33	4.5	Non-acceptable	

Selected samples were four to clarify in biochemical screening and further analysis for identifications.

This Table 2 indicated the summarized form of sample selections and their effect specified on the customer's health. However, there always remains the possibility that contaminants in the juice for consumption can cause infection and that individuals will respond in different ways to a specific strain. The salesman, juice handler will need to carefully assess the safety and efficacy of all new species and strains of microbes before incorporating them into food products.

Graphical studies on migration of pathogens in automated analysis

The case report was presented to assure the security of food safety for all consumes. Now the public health concerns are out of the laws. The government and the people should be concerned to abide by the curriculums based on an ordinance of public health welfare. The awareness campaign is essential to explain by graphical scenario for food safety (Subhasree 2013; Vignaswari 2020). The continuous exposures in street juice and the perishable toxin are responsible for biochemical degradation of juice components and indigestion in human health.

In Fig 3.1, it was clearly stated that the degradations of bio-chemical potency were capable to denature by *E. coli.* within a short course of time. These concentrated values were performed in high level of raising potency of fluid dynamics parallel to decrease of nutritional concentration.

Following the Fig 3.2, it was clearly stated and compared that the degradations of bio-chemical potency were capable to denature in moderate form by *Klebsiella sp.* than *E. coli.* without enhancing any course of time. These concentrated values were degraded slowly and performed in moderate level of raising potency of fluid dynamics parallel to minimize the nutritional components.

The comparative graphical models were diagrammed for decorating the activities of different pathogens and their invasive toxins utilizing carbohydrate and degradations of bio-nutrients along with their fundamentals. So according the graphical model:

Invasiveness = *E. coli.* > *Klebsiella sp.* > *Staphylococcus sp.*

Compared with Test substrate = All are equal in quantities to degrade.

Carbohydrate fermentation =

E. coli., Klebsiella sp.> *Staphylococcus sp.*

Enzymatic fermentation =

Staphylococcus sp.> *E. coli.* > *Klebsiella sp.*

Outcomes

Hand-washing and sanitation do not work due to the resistant nature of the contaminants. Hospitalization rates in countries with high in food poisoning, dehydration and flatulent gastric pain which may occur due to poor sanitation of preparing juice in manual on-road (Table 1, 2). Handwashing can reduce the spread of the infection, but this is an ineffective means of eliminating it (Fig 1.1 and 1.2). A study was reviewed that after 24-48 hours incubation period (Table 3, 4), these infectious pathogens were followed by an abrupt onset of vomiting and diarrhea. Later on, these could be

Table 2: Screening of samples in cultural and biochemical characteristics of applied methods.

Dilution samples	Cultural characteristics				Biochemical test			Inference
	Nutrient agar (Colonies)	MacConkey Agar (Colonies)	PDA agar (Colonies)	BLOOD agar (Hemo-Lysis)	Urease Test (+)	VP Test (+)	Baci-tracing disc (-)	
MS6	+++	+++	+	-	+	+	-	GNB / GPC
DS15	+++	++	+	-	+	+	-	GNB / GPC
FS24	+++	++	+++	-	+	-	-	Fungal colonies
KS27	+++	++	-	+	-	-	-	GNB / GPC

Table 3: Tabular form of isolated findings from screened and categorized samples.

Sampling sites	Samples	Isolated reading from Vitec-2 and compared with Bergey's Manual of Determinative Bacteriology - 8th ed. (Buchanan and Gibbons, 1974) and 9th ed. (Holt *et al.,* 1984)	Culture from Saboured Dextrose Agar
		Bacteria	Fungus
Ganeshguri	Sample 1	*Contaminants in Mixed culture*	-
	Sample 2	*Contaminants in Mixed culture*	-
	Sample 3	*Contaminants in Mixed culture*	-
Malegaon	Sample 4	*Contaminants in Mixed culture*	-
	Sample 5	*Contaminants in Mixed culture*	-
	Sample 6	*E. coli*	-
Jalukbari	Sample 7	*Contaminants in Mixed culture*	-
	Sample 8	*Contaminants in Mixed culture*	-
	Sample 9	*Contaminants in Mixed culture*	-
GMC	Sample 10	*Contaminants in Mixed culture*	-
	Sample 11	*Contaminants in Mixed culture*	-
	Sample 12	*Contaminants in Mixed culture*	-
Dispur	Sample 13	*Contaminants in Mixed culture*	-
	Sample 14	*Contaminants in Mixed culture*	-
	Sample 15	*Klebsiella pneumonia ssp pneumonie*	-
Ulubari	Sample 16	*Contaminants in Mixed culture*	-
	Sample 17	*Contaminants in Mixed culture*	-
	Sample 18	*Contaminants in Mixed culture*	-
Panbazar	Sample 19	*Contaminants in Mixed culture*	-
	Sample 20	*Contaminants in Mixed culture*	-
	Sample 21	*Contaminants in Mixed culture*	-
French Bazar	Sample 22	*Contaminants in Mixed culture*	-
	Sample 23	*Contaminants in Mixed culture*	-
	Sample 24	*Contaminants in Mixed culture*	*Candida sp.*
Khanapara	Sample 25	*Contaminants in Mixed culture*	-
	Sample 26	*Contaminants in Mixed culture*	
	Sample 27	*Staphylococcus aureus*	
Sixmile	Sample 28	*Contaminants in Mixed culture*	
	Sample 29	*Contaminants in Mixed culture*	
	Sample 30	*Contaminants in Mixed culture*	

migrated for urinary tract infections; a low-grade fever may also be present due to secondary infection (Fig 3.1, 3.2, 3.3 and 3.4). On graphical presentation *Klebsiella sp.,* migration rates carry a higher opportunity to take palace in urinary tract infections which comes out reflected analysis of well diffusion technique by automated analysis (Fig 2.5). On the contrary rest of the contaminants (Fig 3.4 and 3.5) were also deadly significant to food toxicity for consumers.

Prevention and control

1. A salesman should work with gloves.
2. The only selected piece should be placed on a machine whereas others should be kept in unsliced.
3. Juice processing should be done in hygienic places.
4. Hygienic ice cubes and water should be processed in plastic glass using for one time.
5. The machine van should be covered by glass for processing.

Table 4: Biochemical analysis of microbial enzymatic reaction values from automated screening.

Test organisms: MS6

Test substrate	Abbreviation	Well	Amount/well (mg)	*Escherichia coli* McFarland:0.61
Ala-Phe-Pro Anylamidase	APPA	2	0.0384	-
ADONITOL	ADO	3	0.1875	-
Alpha D Glucosidase	AGLU	41	0.036	-
Glutamyl Arylamidase pNA	AGLTp	12	0.0324	-
Alpha Galactosidase	AGAL	44	0.036	+
Beta D Glucopyranosidase	BGLU	17	0.036	-
Beta N Acetyle glucosaminidase	BNAG	11	0.0408	-
Beta D Xylosidase	BXYL	21	0.032	-
BETA -GALACTOSIDASE	BGAL	9	0.036	+
Beta Alanine arylamidase pNA	BAlap	22	0.0174	-
Beta Glucoronidase	BGUR	57	0.0378	+
Citrate Sodium	CIT	36	0.054	-
COUMARATE	CMT	56	0.126	+
D Maltose	d MAL	18	0.3	+
D TAGTOSE	d TAG	34	0.3	-
D Mannitol	d MAN	19	0.1875	+
D TREHALOSE	d TRE	35	0.3	+
D Glucose	d GLU	13	0.3	+
D Mannose	d MNE	20	0.3	+
D Sorbitol	d SOR	32	0.1875	+
D Cellulose	d CEL	7	0.3	-
Ellman	ELLM	62	0.3	+
Glycine Arylaminidase	Gly A	46	0.012	-
Glu-Gly-Arg- Arylamidase	GGAA	59	0.0576	-
Gamma Glutamyl Transferase	GGT	14	0.0228	-
Hydrogen Sulphide	H_2S	10	0.0024	-
L Lactate alkalinization	l LATK	40	0.15	-
L Malate Assimilation	l MLTa	61	0.042	-
L ARBITOL	l ARL	5	0.3	-
L Histidine assimilation	l HLSa	53	0.087	-
L Lactate assimilation	l LATa	64	0.186	-
Lysine Decarboxylase	LDC	48	0.15	+
Lipase	LIP	26	0.0192	-
Malonate	MNT	37	0.15	-
N-acetylgalactosaminidase	NAGA	43	0.0306	-
O/ 0129 Resistance	O129R	58	0.0105	-
Ornithine Decarboxylase	ODC	47	0.3	+
Fermentation of Glucose	OFF	15	0.45	+
Phosphates	PHOS	45	0.0504	-
Palatinose	PLE	27	0.3	-
L-Pyrrolydonyl Arylamidase	PyrA	4	0.018	-
L-Proline Arylamidase	ProA	23	0.0234	-
Saccharose	SAC	33	0.3	-
Succinate	SUCT	42	0.15	-
Tyrosine Arylamidase	Tyr A	29	0.0276	-
Urease	URE	31	0.15	-
5 Keto D Gluconate	5KG	39	0.3	+

Table 4: Continues...

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Test organisms: DS15

Test substrate	Abbreviation	Well	Amount/well (mg)	*Klebsiella pneumoniae ssp pneumoniae* McFarland:0.61
Ala-Phe-Pro Anylamidase	APPA	2	0.0384	-
ADONITOL	ADO	3	0.1875	+
Alpha D Glucosidase	AGLU	41	0.036	-
Glutamyl Arylamidase pNA	AGLTp	12	0.0324	-
Alpha Galactosidase	AGAL	44	0.036	+
Beta D Glucopyranosidase	BGLU	17	0.036	+
Beta N Acetyle glucosaminidase	BNAG	11	0.0408	-
Beta D Xylosidase	BXYL	21	0.032	+
BETA -GALACTOSIDASE	BGAL	9	0.036	+
Beta-Alanine arylamidase pNA	BAlap	22	0.0174	-
Beta Glucoronidase	BGUR	57	0.0378	-
Citrate Sodium	CIT	36	0.054	+
COUMARATE	CMT	56	0.126	-
D Maltose	d MAL	18	0.3	+
D TAGTOSE	d TAG	34	0.3	+
D Mannitol	d MAN	19	0.1875	+
D TREHALOSE	d TRE	35	0.3	+
D Glucose	d GLU	13	0.3	+
D Mannose	d MNE	20	0.3	+
D Sorbitol	d SOR	32	0.1875	+
D Cellulose	d CEL	7	0.3	+
Ellman	ELLM	62	0.3	-
Glycine Arylaminidase	Gly A	46	0.012	-
Glu-Gly-Arg- Arylamidase	GGAA	59	0.0576	-
Gamma Glutamyl Transferase	GGT	14	0.0228	+
Hydrogen Sulphide	H_2S	10	0.0024	-
L Lactate alkalinization	l LATK	40	0.15	+
L Malate Assimilation	l MLTa	61	0.042	-
L ARBITOL	l ARL	5	0.3	-
L Histidine assimilation	l HLSa	53	0.087	-
L Lactate assimilation	l LATa	64	0.186	-
Lysine Decarboxylase	LDC	48	0.15	+
Lipase	LIP	26	0.0192	-
Malonate	MNT	37	0.15	-
N-acetylgalactosaminidase	NAGA	43	0.0306	-
O/ 0129 Resistance	O129R	58	0.0105	+
Ornithine Decarboxylase	ODC	47	0.3	-
Fermentation of Glucose	OFF	15	0.45	+
Phosphates	PHOS	45	0.0504	+
Palatinose	PLE	27	0.3	+
L-Pyrrolydonyl Arylamidase	PyrA	4	0.018	+
L-Proline Arylamidase	ProA	23	0.0234	-
Sacharrose	SAC	33	0.3	+
Succinate	SUCT	42	0.15	+
Tyrosine Arylamidase	Tyr A	29	0.0276	+
Urease	URE	31	0.15	+
5 Keto D Gluconate	5KG	39	0.3	-

Table 4: Continues...

Table 4: Continues...

Test organisms: KS27

Test substrate	Abbreviation	Well	Amount/well (mg)	*Staphylococcus aureus* McFarland:0.62
Ala-Phe-Pro Anylamidase	APPA	2	0.0384	-
ADONITOL	ADO	3	0.1875	NA
Alpha D Glucosidase	AGLU	41	0.036	+
Glutamyl Arylamidase pNA	AGLTp	12	0.0324	NA
Alpha Galactosidase	AGAL	44	0.036	-
Beta D Glucopyranosidase	BGLU	17	0.036	NA
Beta N Acetyle glucosaminidase	BNAG	11	0.0408	NA
Beta D Xylosidase	BXYL	21	0.032	NA
BETA -GALACTOSIDASE	BGAL	9	0.036	-
Beta Alanine arylamidase pNA	BAlap	22	0.0174	NA
Beta Glucoronidase	BGUR	57	0.0378	-
Citrate Sodium	CIT	36	0.054	NA
COUMARATE	CMT	56	0.126	NA
D Maltose	d MAL	18	0.3	+
D TAGTOSE	d TAG	34	0.3	NA
D Mannitol	d MAN	19	0.1875	+
D TREHALOSE	d TRE	35	0.3	+
D Glucose	d GLU	13	0.3	NA
D Mannose	d MNE	20	0.3	+
D Sorbitol	d SOR	32	0.1875	-
D Cellulose	d CEL	7	0.3	NA
Ellman	ELLM	62	0.3	NA
Glycine Arylaminidase	Gly A	46	0.012	NA
Glu-Gly-Arg- Arylamidase	GGAA	59	0.0576	NA
Gamma Glutamyl Transferase	GGT	14	0.0228	NA
Hydrogen Sulphide	H_2S	10	0.0024	NA
L Lactate alkalinization	l LATK	40	0.15	+
L Malate Assimilation	l MLTa	61	0.042	NA
L ARBITOL	l ARL	5	0.3	NA
L Histidine assimilation	l HLSa	53	0.087	NA
L Lactate assimilation	l LATa	64	0.186	NA
Lysine Decarboxylase	LDC	48	0.15	NA
Lipase	LIP	26	0.0192	NA
Malonate	MNT	37	0.15	NA
N-acetylgalactosaminidase	NAGA	43	0.0306	-
O/ 0129 Resistance	O129R	58	0.0105	+
Ornithine Decarboxylase	ODC	47	0.3	NA
Fermentation of Glucose	OFF	15	0.45	NA
Phosphates	PHOS	45	0.0504	+
Palatinose	PLE	27	0.3	NA
L-Pyrrolydonyl Arylamidase	PyrA	4	0.018	+
L-Proline Arylamidase	ProA	23	0.0234	-
Saccharose	SAC	33	0.3	+
Succinate	SUCT	42	0.15	NA
Tyrosine Arylamidase	Tyr A	29	0.0276	-
Urease	URE	31	0.15	-
5 Keto D Gluconate	5KG	39	0.3	NA

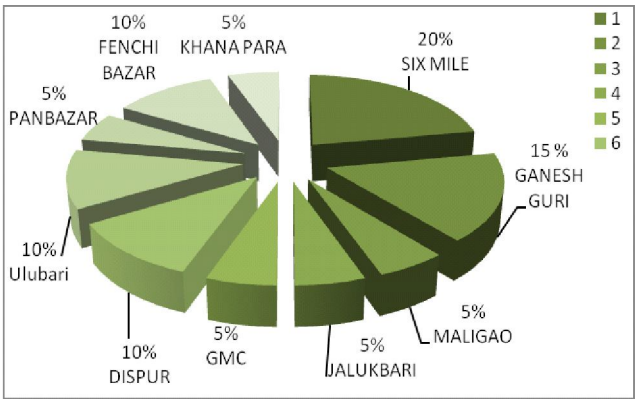


Fig 1.1: Graphical representation on the demography of sales corner in Gauhati city.

Chemical process to neutralize bacterial contaminants

- One spoon black salts.
- Addition of lemon juice.
- Washing of machinery apparatus with 1% acetic acid. (Vinegar) at least five times.

Nearly every scientific paper concerning the human health effects associated with unhygienic juice from roadsides but it should be concerned about regarding microbial infection among consumers. But this particular research was highlighted on simple featuring to emphasize the effect of the drinking sugarcane juice from a street salesman in metropolitan areas respective to human health analyzed on the isolation of different bacterial and fungal components. According to my study, different colonies of fungi and bacteria were isolated and characterized by the

a. 

b. 

Fig 1.2: Remedies for prevention and control of contaminants (a. Removal of extract in polypropylene bags b. Juice additives to neutralize contaminants).

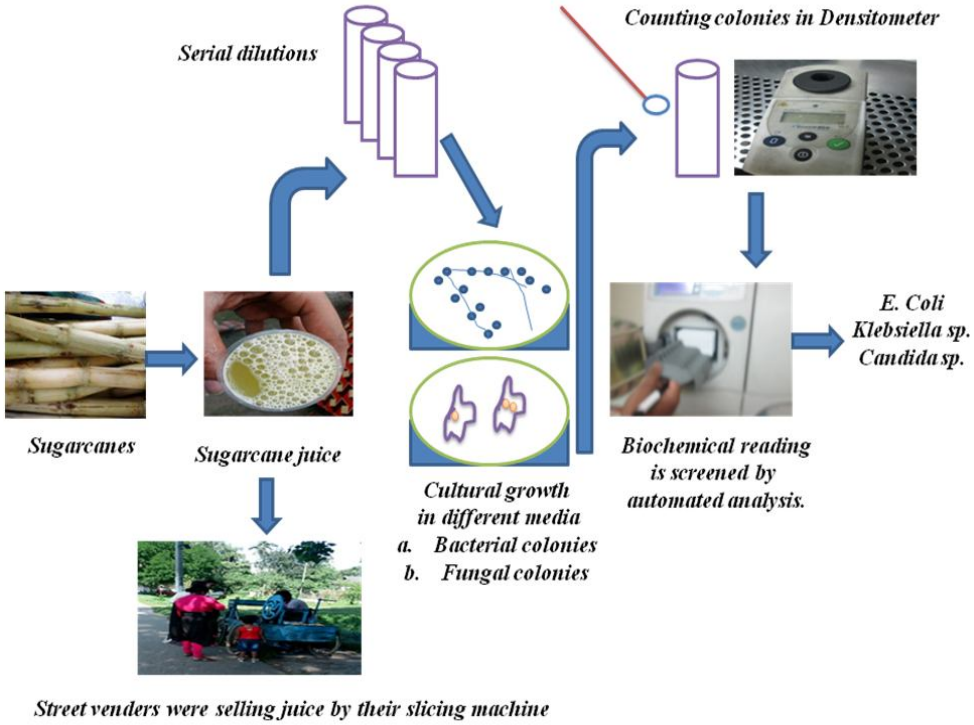


Fig 2.1: Working flow chart.

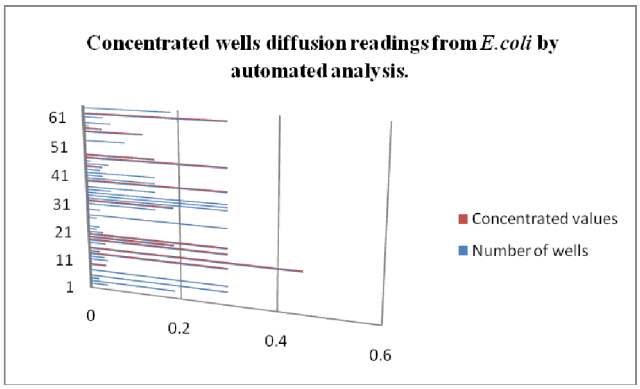


Fig 3.1: Reading values of biochemical degradations of *E. coli* by well diffusion technique in automated analysis.

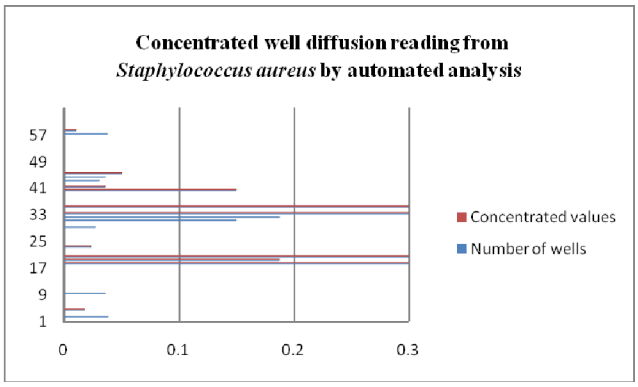


Fig 3.3: Reading values of biochemical degradations of *Staphylococcus aureus* by well diffusion technique in automated analysis.

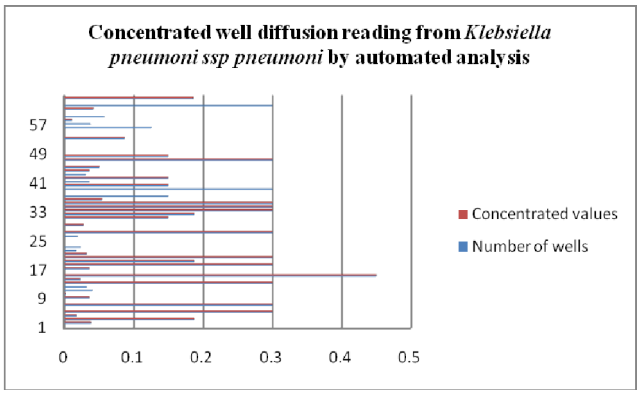


Fig 3.2: Reading values of biochemical degradations of *Klebsiella pneumoniae ssp pneumoniae* by well diffusion technique in automated analysis.

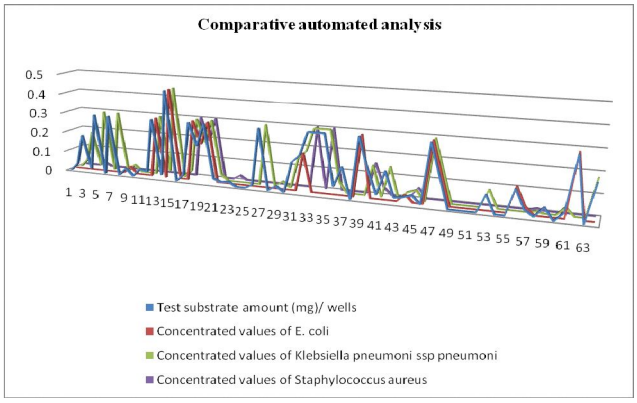
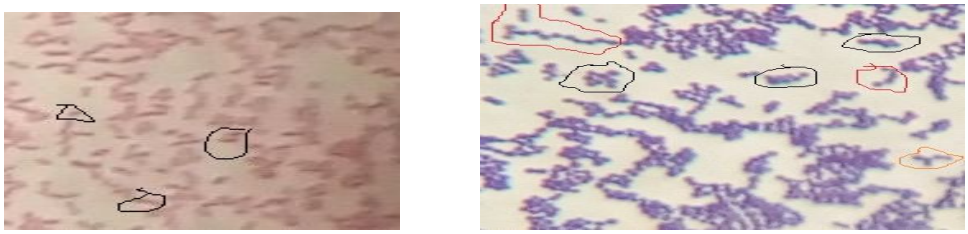


Fig 3.4: Analysis of comparative reading on values of biochemical degradations of *E. coli*, *Klebsiella pneumoniae and Staphylococcus aureus*.



Klebsiella sp.

Red marking: *Streptococcus sp.*
Black marking: *Staphylococcus sp.*

Fig 3.5: Identified gram-negative *bacilli* and gram-positive *cocci* from sugarcane juice.

sample. Samples consist of more bacterial contaminants and mixed colonies then beverage or manufactured. No beverage or manufactured sugar cane juice was found in the local market of Assam. On the contrarily isolates from roadside juice were *Candida sp.,* somehow several samples were mixed with bacterial colonies (*Staphylococcus aureus* and *Streptococcus sp*.). Sugarcane juice extraction easily manipulated by contaminants from aerosol that contain endotoxin, peptidoglycan and various fungal constituents. And the migration of *Klebsiella sp.* as contaminants proves high potency to be virulent rather than others. The prevalence of consumption of sugarcane juice from the street salesman is seen to be more among people of rural areas with very little information available about the concentration of toxic agents present in them. This knowledge may help to explain the high prevalence of gastrointestinal disorders such as diarrhea and food poisoning since these symptoms are also commonly associated with exposure to bioaerosols. Public awareness that sugarcane juice from unhygienic place contains a high concentration of bacterial and fungal constituents may contribute to injurious for health.

The multidisciplinary ulcerative patient should have

cared about lactose or sucrose-containing street-side juices for example sugarcane extracted. Patients are suffered at urinary tract infections may lead to developing urolithiasis, cystitis, ulcerative colitis and cellular eruptions with painful bleeding.

Sugarcanes stems are always susceptible to fungus spores by pollinated transference from other affected plants. On these circumstances "Red rot" diseases are very common affected by *Collecttotrichum falcatum.* So sliced and extracted sugarcane juice is very much vulnerable to carry out mashed toxin (Aflatoxin) as transfusion to the human body. Toxin with secondary bacterial metabolites will promote the decrease level of gut pro bio and enhances the dehydrated thrust to drinkers. Many times it leads to polyurea and ketosis in the body after the accomplishment of fermentation of sugar in cellular transfusion.

CONCLUSION

Unhygienic processing of sugarcane juice from the roadside is threatening for health status and a generalized way to develop the bacterial and fungal related disease in the body. Food concerning and food safety from roadside origin and venders should be clarified by government hands cape. The developing molecular protocols will be modified for further analysis following this specific participating at research in the future for public health significance. The applied research protocols were carried for the further studies based on in-silico analytical findings to find out probiotic against UTI pathogens in humans.

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Conflict of interest

Authors declared no conflict of interest to publish the manuscript on supporting the research data and theorem.

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