Research Article

Study of Antibacterial Activity of Palm Wine against Bacterial Pathogens Isolated From the Clinical Samples.

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Received: 29-05-2017; **Revised:** 15-07-2017; **Accepted:** 11-08-2017.

ABSTRACT

The alcoholic beverages in the world, such as beer, wine champagne etc., are usually made by fermentation with yeasts. The most commonly used organisms are strains of *Saccharomyces cerevisiae*. In many tropical areas of the world, other types of alcoholic beverages are very popular and widely used; these consist of plant saps undergoing a mixed fermentation, containing bacteria from the genus *Zymomonas*. Some of the fermentation products are found very useful for the treatment of various diseases ranging from chronic enteric metabolic disorders to gynecological infections. Palm wine is also called Palm Toddy,it is an alcoholic beverages created from the sap of various species of palm tree. Study was carried out for isolation of bacteria from palm wine and check antimicrobial activity of palm wine against pathogenic bacteria. In current research the bacterial pathogens were isolated from the clinical samples.The antibacterial activity of palm wine carried out by using *pseudomonas species* (20mm zone diameter) and *Klebsiella species*. Palm wine shows higher antibacterial activity against *Klebsiella species* (23mm zone diameter)

Keywords: Palm Wine, *Zymomonas mobilis,* antibacterial activity, Pathogenic bacteria.

INTRODUCTION

The alcoholic beverages in the world such as beer, wine champagne etc. are usually made by the fermentation with yeasts. The most commonly used organisms are the strains of *Saccharomyces cervisiae*. In many tropical areas of the world other types of alcoholic beverages, are very popular & widely used these consist of the plant saps undergoing a mixed fermentation containing bacteria from the genus *Zymomonas.* Some of its fermentation products are found useful in the treatment of various diseases ranging from chroniceneric metabolic disorders to gynocolical infections (swings and De ley 1977) Palm wine is also called Palm toddy, it is an alcoholic beverages produced from the sap of various species of palm tree such as the Palmyra and the coconut palms. This drink is common in various parts of the Asia & Africa and it is known by various names such as

State/territory/ Regions in India	Common Name used
Andhra Pradesh , Kerala	Kallu
Bihar Assam	Tadi
Tamil Nadu	Padaneer
Maharashtra	Tadi, Shindhi

The qualitative characteristic of palm wine depends on the factors such as the period of tapping the palm trees, the palm species, the storage period and the season etc. The concentration of alcohol in palm wine ranges from 0.1 to 7.1 % ethanol, depending on the stages at which it is collected. A normal palm wine contains 4 to 5 % ethanol and has a pH of 3 to 4. The alcoholic fermentation of the sap starts in the collection ground or location. In pats of India, the unfermented sap is called "Neera" and is refrigerated, stored and distributed by semi-governmental agencies. A little lime is added to the sap to prevent it from fermenting. Palm sap contains nutritionally important components including amino acids, Proteins, vitamins, Sugars and micronutrients. Palm sap beings fermenting immediately after collection, due to natural micro flora in the air. Within two hours, fermentation yields an aromatic wine of up to 4 % alcohol.

MATERIALS AND METHODS

Collection of Sample

The sample collected from near Akluj area region at morning time in the sterile plastic cans. The samples were kept in clean dry bottles previously washed and rinsed by distilled water. The sample was then stored at 4^0 C and it was used for further studies.

Isolation of bacteria from palm wine

To isolate inherent bacteria from collected sample requires a specific media, MYPG. It was designed and used. The designed media consists the components all in percentage, (wt/vol) malt extract-0.3; yeast extract- 0.3; glucose- 2; peptone- 0.5. and the pH of media was adjusted to 4.8.

Enrichment of Collected Sample

1 liter broth of designed media was prepared and sterilized by autoclaving at 121°C for 20 minutes. 10 ml sample of collected sap enriched in the suitable detection broth medium and incubated at 30°C for 48 hours. Further isolation, characterization and identification of

pure culture were carried out on agar medium by spread plate technique.

Identification of *zymomonas* species

Colonies suspected to be those of *Zymomonas* were isolated from the enriched sap sample by spread plate and purified by streaking on freshly prepared media incubated for 2 days at 30°C in the anaerobic jar. Isolates from such plates were subjected for the Grams nature, motility and morphological characterization. The biochemical characterization of isolates involves following tests catalase, oxidase, nitrate reduction, indole production and, carbohydrate fermentation.

Biochemical characterization study

Biochemical tests were done for identification of bacteria.

Estimation of alcohol concentration from palm wine

Qualitative estimation of ethanol in an palm wine carried out by using K_2 Cr_2O_7.The various concentrations of alcohol such as 1%,2%.upto 10%prepared.and sequentially filled in the series of test tubes add .1.5 ml of K_2 Cr_2O_7 reagent and incubate at 60 for 30 minutes and recorded. O.D. at 600nm.While palm wine used as unknown.

Isolation and Identification of Pathogenic Bacteria from clinical sample

The clinical samples of patients such as urine were collected from the hospitals of Barshi city. The collected sample was enriched in sterile MacConckeys broth at 37°C for 24 hours. The potential pathogens were isolated on MacConckeysagar plates incubated at 37°C for 24 hours. Isolates from such plates were subjected for the Grams nature, motility and morphological characterization. The biochemical characterization and identification of pathogenic bacterial species from urine samples carried out by using VITEK-2 Autoanalyzer.

Antibacterial activity of palm against the same pathogenic bacteria

Antibacterial activities of palm wine against pathogenic bacteria were studied by agar well diffusion assay. 100µl suspension of isolates of clinical sample was spread with the help of glass spreader on sterile nutrient agar plate. The wells were prepared on sterile nutrient agar with the help of cork borer and each well poured by 100µl palm wine with the help of micropipette. The plates were incubated at 37 ^{0}C for 24 hours.

RESULTS AND DISCUSSION

Isolation of inherent bacterial species from palm wine

When palm wine sample spread on sterile MYPG agar medium and incubated at 30^{o}C for 48 hours. After incubation four isolates were selected randomly for further study.

Morphological Characteristics of Isolates:

Colony characteristics of Isolates isolated on MYPG agar medium incubated at 30°C for 48 hours.

The results are shown in below tables.

Table 1: Colony characters of bacteria isolated from palm wine

Isolate	Morphology	Gram Nature	Motility
1	Rod Shaped	Gram Negative	Motile
2	Rod Shaped	Gram Negative	Motile
3	Cocci	Gram Positive	Motile
4	Rod Shaped	Gram Negative	Motile

Table 2: Gram nature and motility

Isolate	Size(mm)	Shape	Colour	Margin	Elevation	opacity	Consistency
1)	1	Circular	White	Entire	Raised	opaque	Moist
2)	1.5	Circular	White	Entire	Flat	Opaque	Moist
3)	1	Circular	White	Entire	Flat	Opaque	Moist
4)	1.1	Circular	White	Irregular	Raised	Opaque	Moist

Table 3: Biochemical test

Sr. No	TEST	ISOLATES 1	ISOLATES 2	ISOLATES 3	ISOLATES 4
1	Indole Production	-	-	-	-
2	Methyl red test	-	-	-	-
3	Vogues paskaur test	-	-	-	-
4	Citrate utilization test	-	-	-	-
5	Glucose	+	+	+	+

6	Maltose	+	-	+	+
7	Fructose	+	+	+	+
8	Lactose	-	-	-	-
9	Oxidase	-	-	-	+
10	H2S Production	-	-	-	-

Table 4: Estimation of palm wine by K2Cr2O7

Sr.No.	Conc.of alcohol (%)	Amount of alcohol in Ml	Amount of D/W in Ml	K2Cr2O7 Reagent in Ml	Incubation	O.D.at 600nm
1.	1.	1	1	1.5		0.20
2.	2.	1	1	1.5	In water bath at 60^{0}C for 30 Minutes	0.30
3.	3.	1	1	1.5		0.60
4.	4.	1	1	1.5		0.80
5.	5.	1	1	1.5		0.140
6.	6.	1	1	1.5		0.150
7.	7.	1	1	1.5		0.160
8.	8.	1	1	1.5		0.180
9.	9.	1	1	1.5		0.200
10	10	1	1	1.5		0.235
11	Unknown	0.5	05	1.5		0.40
12	Blank	-	1	1.5		0.0

Isolation and Identification of bacterial pathogens from urine sample

The clinical sample spread on MacConkeys agar medium after incubation their morphological and biochemical characterization study was done

Identification of *Pseudomonas* species on Vitek -2 (Autoanalyzer)

Identification Information	Analysis Time:	8.00 hours	Status:	Final
Selected Organism	97% Probability Bionumber:	Pseudomonas aeruginosa 0003041103500352		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	+	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	+			

Identification of *Klebsiella* species on Vitek -2 (Autoanalyzer)

Identification Information	Analysis Time:	10.00 hours	Status:	Final
Selected Organism	90% Probability Bionumber:	Klebsiella pneumoniae ssp pneumoniae 6637735753567152		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	+	53	IHISa	+	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	+			

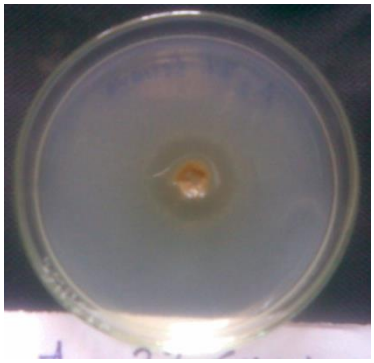

Photo 1: Antibacterial activity of palm wine against *Klebsiella*species

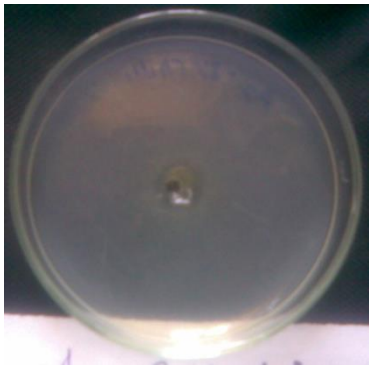

Photo 2: Antibacterial activity of palm wine against *Pseudomonas* species

Antibacterial activity of palm wine

Sr. No.	Pathogenic Bacteria	Inhibition zone in diameter
1	*Pseudomonas* species	20mm
2	*Klebsiella* species	23mm

Antibacterial activity of palm wine against *Pseudomonas* and *Klebsiella* species

CONCLUSION

The palm wine consist inherent flora such as organisms studies on the basis of morphological, cultural and biochemical characteristics. Amongst *Zymomonas spp*. were found to be predominant.

The antibacterial activity of Palm wine studied against the pathogens like *Pseudomonas* species and *Klebsiella* species by agar well diffusion method. The sample of palm wine showed higher antibacterial activity against *Klebsiella* species (23mm inhibition zone diameter) and *Pseudomonas s*pecies (20mm inhibition zone diameter).

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Source of Support: Nil, **Conflict of Interest:** None.