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Study of Microbial and Anti-microbial Properties of Palm Wine

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ABSTRACT

Palm wine is a traditional alcoholic drink and act as an excellent substrate for microbial growth, which was obtained from the sap of various types of palm trees. The pH and alcoholic content of the fresh palm wine were determined as 7.2% and 0.09%. Yeast, aceticacid and lactic acid bacteria were isolated from fresh palm wine by pour plate technique using suitable selective media by providing optimum growth conditions. The morphological and biochemical characterization identifies the cultures as Saccharomyces, Lactobacillus and Acetobacter sp. Anti-microbial activity of yeast and Lactic acid bacteria was determined by performing diffusion assays. Among the two organisms yeast produced significant Anti-microbial activity with 26 mm of inhibition zone diameter against Staphylococcus aureus, and 22 mm of inhibition zone diameter against Pseudomonas aeroginosa.

Keywords: Palm wine, Biochemical properties, Anti-microbial activity, *Saccharomyces*, *Lactobacillus*.

INTRODUCTION

Palm wine is the fermentation product of sap collected from trees of *Palmae* family [1]. It is having two properties one is responsible to give sweet taste immediately after its collection and good nutritional value which plays a significant role in

medical field to fight against pneumonia, to induce breast milk in mothers, rich in vitamins and aminoacids, improves eye health. On the other hand over fermented palm wine results in production of diarrhoea, hernia, and headaches, liver disorders [2]. Palm wine is rich in sugars to support the growth of several microorganisms. Bacteria and yeast are the dominating organisms reduce the sugar substrates into different metabolites like acetic acid, lactic acid, ethanol [3,4]. Yeasts, Lactic acid and acetic acid bacteria are mainly responsible for fermentation and for production of palm wine. There are several reports on anti-microbial activity of palm wine against bacteria and fungi [5,6]. An attempt was made in the present work to isolate yeast, lactic acid and acetic acid bacteria from palm wine and to determine the Anti-microbial activity against some pathogenic organisms.

MATERIALS AND METHODS

Palm wine collection

Previously sterilized and cooled glass bottles were used for collection of sample directly from the incisions of the tree trunk. It was transported immediately to the laboratory in 30 min.

Biochemical nature of palm wine

pH: pH of the freshly collected palm wine sample was determined by directly by keeping the electrode in sample. The pH meter (Hanna precision pH meter, Model pH 213) was calibrated with phosphate buffer pH 7.

Acidity of the sample: The acid content of the sample was determined by titrating with 0.1 N NaOH solution using phenolphthalein as indicator [7].

Ethanol content: Ethanol content in palm wine sample was estimated by spectroscopic method [8]. After completion of reaction the samples colour was read at 578 nm and the percentage of ethanol calculated using the following formula:

$$\text{Percentage of ethanol in sample (\%)} = (C_1/C_2)\,(A_1/A_2) \times 100$$

Where C_1 = Concentration of standard, C_2 = Concentration of sample as per labeled amount,

A_1 = Absorbance of standard, A_2 = Absorbance of sample.

Determination of microbial load

Total microbial content of the fresh sample estimated by diluting 1 mL of sample with 9 mL of sterile water to produce 10^{-1} to 10^{8} dilutions. From each dilution 1mL of sample transferred to the sterile media (Table 1) to isolate yeast, Lactic acid and Acetic acid bacteria by pour plate method. Plates for isolation of yeast incubated at a temperature of 30°C for 4-5 days and for aceticacid and lactic acid bacteria at a temperature of 37°C for 2 days. Colony forming units/mL was counted by digital colony counter.

Table 1: Composition of media for enumeration of yeast, lactic acid and acetic acid bacteria.

Ingredients	**Weight in g/ 100 mL for isolation of**		
	Yeast	**Lactic acid bacteria** (Deman Rogosa Sharpe Agar**)**	**Acetic acid bacteria** (Yeast extract, peptone, mannitol medium)
Yeast extract	0.5	0.25	0.5
Glucose	0.2	0.1	-
Peptone	-	0.5	0.3
Mannitol	-	-	2.5
Cycloheximide	-	0.01	-
Chloramphenicol	0.01	-	-
Agar	2	2	2
pH	7.6 ± 0.2	7.4 ± 0.2	5.8 ± 0.2

Microscopic and biochemical characterization [9]

Microscopy: Simple and gram's staining were used for identification of bacteria. Simple staining with lactophenol blue was used for yeast.

Biochemical tests for identification of yeast: Carbon assimilation test was used to check the carbohydrate adaptability of yeast isolate and verified by incorporating different carbon sources (glucose, sucrose, maltose, xylose, lactose, mannitol and fructose) in 1% concentration in sterile yeast fermentation base medium. In the same manner nitrogen assimilation test was performed by incorporating nitrogen sources like lysine, potassium nitrate, ammonium nitrate and glutamic acid. Urea hydrolysis, gelatin liquefaction tests were also performed.

Biochemical tests for identification of *Lactobacillus*: Sugar fermentation test, Catalase test, Nitrate reduction test, Sodium chloride tolerance tests were performed.

Biochemical tests for identification of acetic acid bacteria: Productions of acid in 1% of sugar containing medium, catalase tests were performed.

Determination of Anti-microbial activity

***Test organisms*:** 24 h fresh cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were used for assay.

***Method*:** Agar well diffusion method was performed by following Indian Pharmacopoeia 2015 [10]. Broth cultures of yeast and *Lactobacillus* were developed by growing in media mentioned in Table 1, 50 µL of above grown culture broth was added to the respective wells or one single loop of each organism were streaked on the nutrient agar plates with test organism and allowed to diffuse at room temperature for 30 min. The inoculated plates were kept for incubation at 37°C for 24 h in case of lactic acid bacteria and 30°C for 4-5 days for yeast. After incubation, a clear zone was observed around the well which was evidence of the presence of anti-bacterial active compounds in the culture tubes isolated from palm wine. Diameters of the zone of inhibition were measured in millimeters (including the diameter of the well).

RESULTS AND DISCUSSION

Biochemical properties of palm wine

The freshly collected palm wine has 7.2 of neutral pH and very low content of ethanol i.e., 0.09%. Acid content estimated by titrimetric method and exhibited 0.6 ± 0.2. Fresh palm wine has high content of sugars and more microbial load. In the presence of sun light rapid rate of fermentation initiated by microorganisms and consumes all the sugars to produce alcohol. Due to rapid rate of fermentation the acid content increases and drop in pH would observe. In freshly collected palm wine shows almost neutral pH, less amount of ethanol and acid content [11].

Microbial load determination

Pour plate technique was done to count the total microbial population in fresh sample by making serial dilution from 10^{-1} to 10^{-8}. The sample was rich in yeast and Lactic acid bacteria and low in acetic acid bacteria (Figure 1).

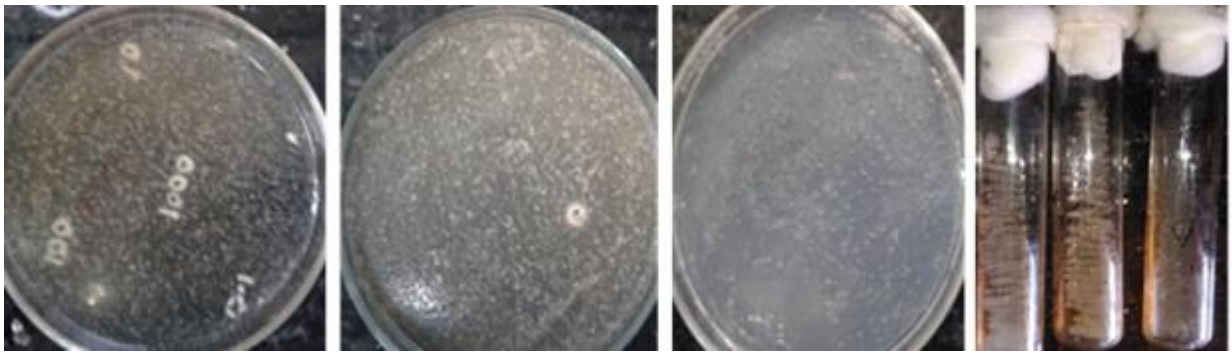

Figure 1: Isolated colonies of yeast, lactic acid and acetic acid bacteria, in agar slants.

Microscopic and biochemical characterization

Isolates were subjected to morphological identification by performing Grams and simple staining. A cream and round shaped colony with raised elevation identifies the culture as yeast organism and it was further confirmed by performing biochemical tests. Yeast is the commonly found organisms in palm wine with different species like *Saccharomyces* responsible for alcohol production. After performing staining techniques *Lactobacillus* was identified as gram positive rods and acetic acid bacteria was identified as gram positive and the colonies were smooth, moist, glistening, cream in colour and spherical in shape (Figure 2).

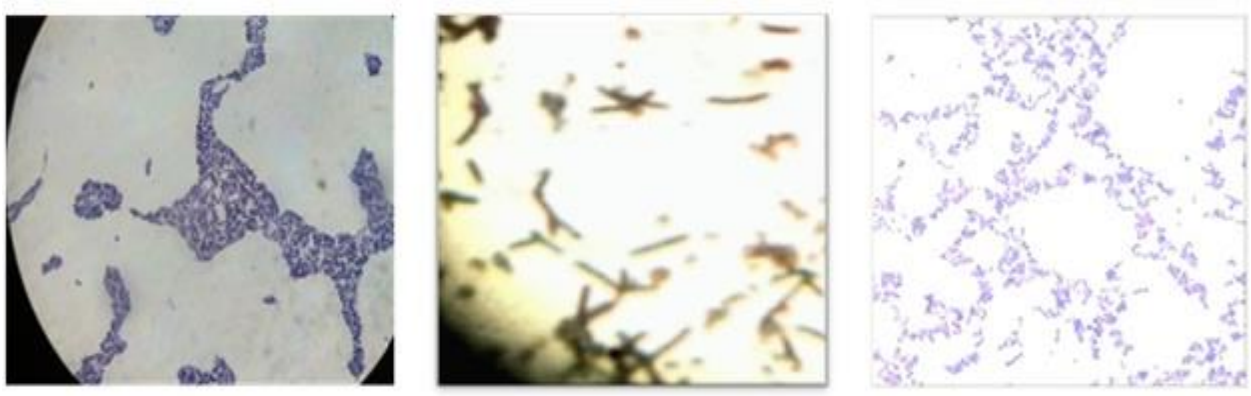

Figure 2: Morphological identification of yeast, lactic acid and acetic acid bacterial.

The isolated organisms have wide range of sugar utilizing capability. Yeast adapted to some sugars like glucose, sucrose, maltose, mannitol and fructose and produced huge growth in media but in the remaining sugars growth was negligible. Because yeast especially *Saccharomyces* doesn't have lactose utilizing enzymes - lactase or β–galactosidase [12]. Nitrogen sources like ammonium nitrate and glutamic acid gives increased growth of yeast and less adaptability towards nitrate and lysine confirms the presence of *Saccharomyces* sp. Further confirmation of *Saccharomyces* by producing negative result with urea hydrolysis and

gelatin liquefaction. Molecular level screening is required to confirm to its genus level. This preliminary identification results gives an idea of presence of *Saccharomyces* in freshly collected palm wine sample. The important organism in palm wine responsible for alcoholic fermentation and for characteristic odorant production is *S. cerevisiae* [13].

Lactic acid bacteria fermented the following sugars like lactose, maltose, sucrose, glucose and fructose and confirms the presence of *Lactobacillus* sp. Negative catalase and nitrate reduction tests, good tolerance with sodium chloride confirms *L. plantarum* as these organisms are predominate in palm wine sample under lactic acid bacteria category but, molecular level of evidence also required for further confirmation

Biochemical tests for identification of Acetic acid bacteria

No acid production in glucose, fructose, sucrose, mannitol, glycerol and positive catalase reaction confirms the *Acetobacter* sp., of acetic acid bacteria. Among the acetic acid bacteria *Acetobacter* and *Gluconobacter* sp., are the predominant in number [13].

Anti-microbial activity

Anti-microbial activity of the microorganisms isolated from palm wine was determined by streak plate and cup plate method against pathogenic and non-pathogenic bacteria and the results were given in Table 2 and Figure 3 which shows that Yeast isolates are having highest Anti-microbial activity against *S. aureus* and least activity against *B. subtilis* organisms. The anti-bacterial activity of Yeast determined by cup plate and streak plate method (Figure 4). Similarly the anti-bacterial 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 highest anti-bacterial activity against *Klebsiella* species (23 mm inhibition zone diameter) and *Pseudomonas* species (20 mm) [5].

Table 2: Anti-bacterial activity of yeast and lactic acid bacteria.

Isolate	*E. coli* (IZD in mm)	*S. aureus* (IZD in mm)	*B. subtilis* (IZD in mm)	*P. aeruginosa* (IZD in mm)
Yeast	18	26	16	22
Lactic acid bacteria	15	20	18	17

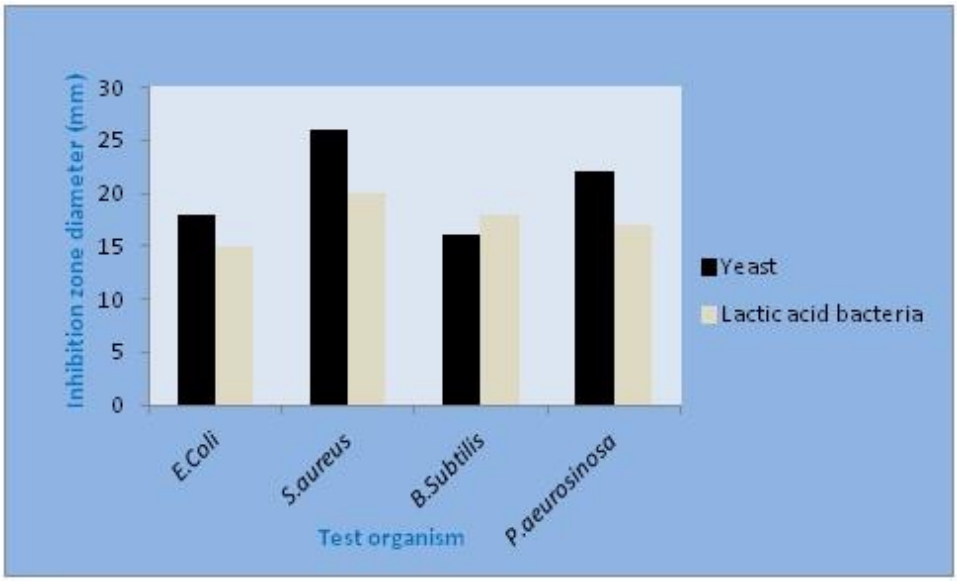

Figure 3: Anti-bacterial activity of yeast and lactic acid bacteria.

Among the two organisms yeast produced significant anti-microbial activity with 26 mm of inhibition zone diameter against S. *aureus*, and 22 mm of inhibition zone diameter against *Pseudomona aeroginosa* (Figure 4).

Figure 4: Anti-bacterial activity of yeast isolated from palm wine.

Lactic acid bacteria exhibited less anti-bacterial activity compared to yeast isolate (Figures 3 and 5). Alcohol produced from yeast may be responsible to show better anti-bacterial activity than Lactic acid bacteria.

Figure 5: Anti-bacterial activity of *Lactobacillus* against *S. a*, *E. coli.*

Lactic acid bacteria exhibited less anti-bacterial activity compared to yeast isolate. *Lactobacillus* showed highest anti-bacterial activity against *S. aureus*, least activity against *E. coli* represented in Figure 3.

Palm wine has remarkable anti-bacterial activity against *S. aureus* and *P. aeroginosa* that are now showing resistance to most antiseptic agents and antibiotics [14]. Alcohol producing ability of Yeast from palm wine is responsible for Anti-microbial activity by denaturation of proteins and lysis of cell membrane. Alcohol content of the palm wine increases as the fermentation time increases due to rapid consumption of sugars in it. Alcoholic compounds have bactericidal effects on vegetative form bacteria [15]. Thus, ethyl alcohol is frequently used as the base of disinfectants. The bactericidal effect associates with the alcohol's ability in changing the chemical structures of permeability of bacterial cell walls. Alcohol with water combination penetrates the enzyme system of microorganisms; inhibit metabolism, growth and reproduction by targeting the enzymes like dehydrogenase and oxidase [16]. *Lactobacillus* ferment the sugars in palm wine and produces organic aid like lactic acid which reduces the pH of the growth medium to show the Anti-microbial activity [17]. Low pH of the lactic acid soluble in lipids there by facilitates the diffusion of acid through the cell wall and finally reaches to cytoplasm to cause damage. In addition to Lactic acid production *Lactobacillus* and some other lactic acid bacteria produces hydrogen peroxide, aldehydes and bacterocins to act as anti-microbials [18].

CONCLUSION

Among the two organisms yeast produced significant Anti-microbial activity with 26 mm of inhibition zone diameter against S. *aureus*, and 22 mm of inhibition zone diameter against *Pseudomonas aeroginosa*. Lactic acid bacteria exhibited less anti-bacterial activity compared to yeast isolate. From the previous reports and present study results revealed the palm wine is the alternative source to show disinfectant activity. Because of its sugary nature it is not possible to use in clinical labs or as household disinfectant, it can used in farms at least, to control unnecessary bacterial contaminants.

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