



ANTIBACTERIAL EFFICACY OF PALM WINE AGAINST COMMON BACTERIAL ISOLATES FROM VARIOUS CLINICAL SAMPLES

Microbiology

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ABSTRACT

Palm wine is a traditional alcoholic beverage produced by natural fermentation of the sap of palm trees. This drink is common in various parts of the Asia & Africa and it is known by various common names such as Maharashtra (Tadi, Shindhi). The unfermented sap is called neera (Palghar in Maharashtra) and is refrigerated, stored and distributed by semi-government agencies. This study was conducted to investigate the antibacterial potential of palm wine on as an alternative to antibiotics by studying its antibacterial efficacy against common bacterial isolates from clinical samples. Freshly top-tapped palm wine samples were obtained from a local palm wine tapper in Palghar, Maharashtra state, India. Various strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* was carried out using agar diffusion method. The palm wine used in this assay inhibited the growth of all the selected bacterial isolate used. The inhibition ranged from 2.3 mm- 29.5mm. Palm wine subjected to fermentation at room temperature for 7 days (168 hours). Growth inhibitory effect of undiluted palm wine superior than groups of antibiotics such as Ampicillin, Ampiclox, Chloramphenicol, Tetracycline and Ciprofloxacin. This study established that palm wine possess considerable antibacterial activity against selected common bacterial isolates. Since palm wine has shown potent antibacterial activity on the test bacteria, further clinical trials will be required that will prove the potential of palm wine as an alternative to antibiotics treatment among patients suffering from common infection caused by there organism.

KEYWORDS

Antibiotic Sensitivity Test, Ampicillin, Ampiclox, Chloramphenicol, Tetracycline and Ciprofloxacin

INTRODUCTION

Palm wine is a traditional alcoholic beverage produced by natural fermentation of the sap of palm trees such as include *Elaeis guineensis*, *Raphia regalis*, *Raphia sudanica*, *Raphia vinifera*, *Raphia hookeri* and *Borassus aethiopum*. Palm sap contains nutritionally components including amino acids, Proteins, vitamins, Sugars and micronutrients. It is whitish in colour with different varieties of flavours, ranging from sweet (unfermented) to sour (fermented) and vinegary. however, it is mostly enjoyed when sweet.^[1,2]

The unfermented sap is called neera (Palghar in Maharashtra) and is refrigerated, stored and distributed by semi-government agencies. A lime is added to the sap to prevent it from fermenting. Neera, similar to fruit-juice products, is comparatively rich in potassium. Palm sap contains natural yeasts, which help in the fermentation of glucose to alcohol. Which subsequently converts the alcohol to acetic acid. This drink is common in various parts of the Asia & Africa and it is known by various common names such as Andhra Pradesh and Kerala (Kallu), Bihar Assam (Tadi), Tamil Nadu (Padaneer), Maharashtra (Tadi, Shindhi).^[2,3]^{wikipedia}

The qualitative characteristic of palm wine depends on the factors such as the period of tapping the palm trees species, the storage period and the season. 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 optimum range palm wine contains 4 to 5 % ethanol and has a pH of 3 to 4.^[3-5]

Palm wine plays an important role in many adivasi community ceremonies in Palghar district, Maharashtra such as Varali, Kokana, Ka-Thakur peoples. Palm wine is often infused with medicinal herbs to remedy a wide variety of physical complaints (medical uses) and associated with a decrease in death due to cardiovascular diseases, although highly consumption of palm wine with high alcohol content has adverse health effects.^[1,3-5,8]

This study was conducted to investigate the antibacterial potential of palm wine on as an alternative to antibiotics by studying its antibacterial efficacy against common bacterial isolates from clinical samples.

MATERIALS AND METHODS

This study was conducted in April 2022.

Source of Palm Wine

Freshly top-tapped palm wine samples were obtained from a local palm wine tapper in Palghar, Maharashtra state, India. The samples were collected using sterilized one litre capacity sample bottles with screw caps. This reduces fermentation rate considerably before the palm wine is taken into the laboratory for further studies.^[7,8]

Source of Test Organisms

Various strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were collected from Vedantaa Institute of Medical Sciences, Vedantaa Hospital and Research centre. They were confirmed by using standard laboratory methods.

Standardization of Test Organism

Standardization of the test organisms was carried out by culturing the organisms on nutrient broth for 24 hours, after which they were cold centrifuged (Remi-NEYA 16 R) at 8000 rpm for 5 minutes. The supernatant was then decanted and 10ml of sterile distilled water was added and then re-centrifuged for 5 minutes to get the harvested cells. Serial dilution of the harvested cells was then carried out and plated on nutrient agar and incubated at 37°C for 24 hours, after which the colonies were counted.

Growth Inhibitory Activity of Palm Wine on the Test Organisms

Using the technique of agar well diffusion. 1 ml of standardized cells of each of the test organisms was taken using a sterile syringe and placed into sterile petri-dishes. Each plate was then overlaid with 20 ml Muller- Hinton Agar (Himedia-M173), carefully swirled to allow even distribution of the organisms within the agar and were allowed to gel before 4 wells (8 mm in diameter) were bored within the agar with the help of a sterile cork borer. 0.1 ml of the undiluted and the ten-fold serially diluted palm wine sample (1:10, 1:100, 1:1000, 1:10000) were dispensed into the wells. The plates were incubated at 37°C for 24h. Sterile distilled water was used as control and the plates were incubated at 37°C for 24h. The determined and recorded diameter of the zones of inhibition around the wells containing the palm wine. This assay was repeated every 24h for 7 days to determine the effect of fermentation of palm wine on growth inhibition against the bacterial isolates.

Antibiotic Sensitivity Test

Antibiotic sensitivity test carried out by standard laboratory methods

such as agar well diffusion assay.

Determination of pH

The pH of the palm wine samples used ranged from 6.0-2.0. pH was measured using Auto digital pH meter (Model- Labline 11) after homogenizing 10 ml of the palm wine samples in 40 ml of distilled water. The pH meter was standardized with buffer solution.

Titrate Acidity Determination

The TTA value of the palm wine samples used ranged from 1.0-6.0. For TTA measurement, 10 ml of the palm wine samples was homogenized in distilled water (20ml). followed by addition of 2 drops of phenolphthalein indicator. The sample was then titrated against 0.1M NaOH to an end point of a certain pink colour. The quantity of NaOH used was noted and the titratable acid percentage was calculated using the subsequent formula;

$$\text{TTA}(\%) = V \times 0.15$$

RESULTS

Growth Inhibition of Test Bacteria by Palm Wine

Table 1: Growth inhibitory effect of undiluted palm wine on selected bacterial isolates. Diameters of Zones of Inhibition (mm)

Days of Fermentation	E. coli	S. aureus	P. aeruginosa	K. pneumonia
Day 1	12.3	6.7	15.0	0.0
Day 2	15.8	9.1	16.1	0.0
Day 3	17.1	11.3	18.5	0.0
Day 4	20.0	11.9	19.0	0.0
Day 5	21.5	13.5	21.3	0.0
Day 6	23.4	18.2	23.6	0.0
Day 7	26.6	24.0	29.5	26.0
Ampicillin	0.0	17.2	18.6	0.0
Ampiclox	0.0	14.4	19.3	0.0
Tetracycline	0.0	19.7	24.5	17.4
Chloramphenicol	12.0	20.1	16.3	21.2
Ciprofloxacin	19.0	22.8	26.7	15.3

The palm wine used in this assay inhibited the growth of all the selected bacterial pathogens used. The inhibition ranged from 2.3 mm-29.5mm. Palm wine subjected to fermentation at room temperature for 7 days (168 hours) exerted the greatest inhibitory effect on all the test bacteria while the freshly tapped recorded the least inhibitory effect (Table 1, 2, 3 and 4). Table 1 showed that the zone of inhibition against the test isolates increased in Days 7 when compared with other days. There was no zone of inhibition recorded for Klebsiella pneumonia from Day 1 to Day 6. While in the groups of antibiotics, Chloramphenicol, Tetracycline and Ciprofloxacin were observed to exhibit zones of inhibition against the test isolates when compared with others.

Table 2: Growth inhibitory effect of undiluted palm wine (1:10) on selected bacterial isolates. Diameters of Zones of Inhibition (mm)

Days of Fermentation	E. coli	S. aureus	P. aeruginosa	K. pneumonia
Day 1	5.9	3.6	6.0	0.0
Day 2	7.4	4.2	6.5	0.0
Day 3	9.8	5.5	8.2	0.0
Day 4	10.9	5.8	10.6	0.0
Day 5	12.2	7.3	10.9	0.0
Day 6	13.5	9.1	11.7	0.0
Day 7	14.7	12.4	14.9	13.7
Ampicillin	0.0	17.2	18.6	0.0
Ampiclox	0.0	14.4	19.3	0.0
Tetracycline	0.0	19.7	24.5	17.4
Chloramphenicol	12.0	20.1	16.3	21.2
Ciprofloxacin	19.0	22.8	26.7	15.3

Table 2 and Table 3 showed serially diluted palm wine (1:10 and 1:1,000), zone of inhibition observed against bacterial pathogens were decreased.

Table 3: Growth inhibitory effect of undiluted palm wine (1:1,000) on selected bacterial isolates. Diameters of Zones of Inhibition (mm)

Days of Fermentation	E. coli	S. aureus	P. aeruginosa	K. pneumonia
Day 1	2.5	2.3	3.1	0.0

Day 2	3.1	2.8	3.2	0.0
Day 3	5.3	2.9	4.7	0.0
Day 4	5.9	3.1	5.8	0.0
Day 5	6.6	4.2	6.3	0.0
Day 6	6.9	5.6	6.9	0.0
Day 7	7.2	6.5	8.0	7.1
Ampicillin	0.0	17.2	18.6	0.0
Ampiclox	0.0	14.4	19.3	0.0
Tetracycline	0.0	19.7	24.5	17.4
Chloramphenicol	12.0	20.1	16.3	21.2
Ciprofloxacin	19.0	22.8	26.7	15.3

Table 4: Growth inhibitory effect of undiluted palm wine (1:10,000) on selected bacterial isolates. Diameters of Zones of Inhibition (mm) There was no zone of inhibition recorded against Escherichia coli, Staphylococcus aureus Pseudomonas aeruginosa, Klebsiella pneumonia from Day 1 to Day 7 (Table 4).

Determination of pH

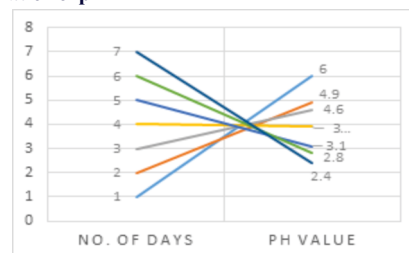


Fig 1: -pH value of fermenting palm wine

The pH of the freshly tapped palm wine was 6.0 but by the 7th day it has decreased to 2.4

Total Titratable Acidity (TTA) of the palm wine samples used

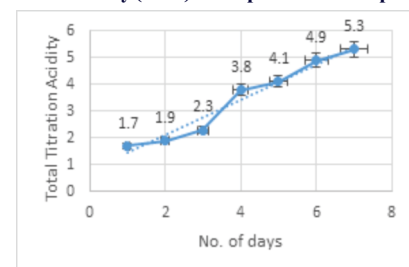


Fig 2: -TTA value of fermenting palm wine

The TTA value of the freshly tapped palm wine was 1.7 but by the 7th day it has increased to 5.3 (fig. 2).

DISCUSSION

In this study, the effect of palm wine (Tadi) on pathogenic bacteria was investigated. The palm wine used inhibited the growth of four test organisms. The highest inhibition was observed with the palm wine subjected to natural fermentation for 7 days on the four organisms used. Akinrotaye et al studied by the effect of palm wine from Raphia palm trees on diarrhoeic bacteria was investigated. The palm wine used inhibited the growth of Staphylococcus aureus, E.coli. The highest inhibition was observed with the palm wine subjected to natural fermentation for 7 days of these organism. Olotu et al reported by the zone of inhibition against the test isolates increased in Days 5, 6 and 7 when compared with other days.

In the study, there was no zone of inhibition recorded for Klebsiella pneumonia from Day 1 to Day 6. While in the groups of antibiotics, Chloramphenicol, Tetracycline and Ciprofloxacin were observed to exhibit zones of inhibition against the test isolates when compared with others. In the study by Olotu et al. the undiluted palm wine inhibited the growth of all selected test organisms and exhibited higher zones of inhibition compared to the serially diluted (1:10, 1:100, 1:1000, 1:10000) samples.

The pH of the freshly tapped palm wine was 6.0 but by the 7th day it's decreased to 2.4. The activities of the array of microorganisms present which metabolize the sugars present in the palm wine to produce

alcohol and organic acid with a help of decrease in pH of the sample. Similar studied, Ekundayo et al, Faparusi et al and Rose et al reported by the drop in pH of palm wine in the first few days from 6.20 to 3.42 might likely be responsible for the inhibition. Rokosu et al and Fooks et al. suggest that, the organic acid produced at this pH is lactic acid, because there were usually a predominance of lactic acid bacteria at this stage of fermentation.

The TTA value of the freshly tapped palm wine was 1.7 but by the 7th day it has increased to 5.3 (fig. 2). Akinrotoyee et al, showed that also revealed are consistent increases in titratable acidity of the palm wines throughout the period of fermentation as shown in which increases from 1.98 to 6.05. Snell et al and Ettre et al reported by the titratable acidity of palm wine is expected to be between 0.5 to 1.0%. Studies have shown that during fermentation of palm wine, high titratable acidity is inhibitory to the growth of spoilage organisms but creates conducive environment for the growth of desirable organisms similar studied Reddy et al.

CONCLUSION

In conclusion, this study established that palm wine possess considerable antibacterial activity against selected common bacterial isolates. The longer the duration of fermentation, the more effective was the palm wine in inhibiting the growth of these test organisms. Since palm wine has shown potent antibacterial activity on the test bacteria, further clinical trials will be required that will prove the potential of palm wine as an alternative to antibiotics treatment among patients suffering from common infection caused by there organism. In view of the increasing antibiotics resistance worldwide, alternative choice to antibiotics may have a significant impact in medicine.

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