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Full Length Research Paper

Antimicrobial property of palm wine

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Palm wine was assessed for its antimicrobial property. The antimicrobial property was assayed on five bacteria: *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aureginosa* and a *Bacillus sp* with one fungus *Candida albicans.* The minimum inhibitory concentration and the minimum bactericidal concentration of palm wine on these organisms were determined using agar well diffusion method. The antimicrobial activity on these organisms was then compared with the action of methylated spirit, a known commercial disinfectant. Palm wine was found to be more active against the gram negative organisms (*Escherichia coli, Pseudomonas aureginosa* and *Proteus mirabilis*) than the gram positive organisms (*Bacillus sp* and *Staphylococcus aureus*). The action was bactericidal on the gram negative organisms while it was bacteristatic on the gram positive. It was found out that palm wine had no inhibitory or cidal action on *Candida albicans* which is one of its resident organism. The antimicrobial action was also found to compare favorably with methylated spirit a known commercial disinfectant.

Keywords: Palm wine, Antimicrobial, Bactericidal, Bacteristatic, Gram negative, Gram positive.

INTRODUCTION

Palm wine generally refers to a group of alcoholic beverages obtained by fermentation from the saps of palm trees Agu et al., (1999). It is a whitish liquid produced by natural fermentation of the sap of *Elaeis guineensis* and *Raphia hookeri* (Uzogara et al., 1990; Uzochukuru et al., 1991). The unfermented sap is clean, sweet, colourless syrup containing about 10 - 12% sugar, which is mainly sucrose (Bassir, 1962; Okafor, 1975a). It is a refreshing beverage widely consumed in Southern Nigeria and other parts of the world particularly Asia and Southern America, Elijah *et al.,* (2010). Although palm wine may be presented in a variety of flavours, ranging from sweet (unfermented) to sour (fermented) and vinegary, it is mostly enjoyed by people when sweet. Elijah *et al.,* (2010). Fresh palm sap is a sweet, clear, colourless juice, which has high sugar content.

Upon fermentation by the natural microbial flora, the sugar level decreases rapidly as it is converted to alcohol and other products Obire, (2005) whereas, the sap becomes milky-white due to the increased microbial suspension resulting from the prolific growth of the fermenting organism (Okafor, 1975a,b). Generally, both brands of palm wine have several nutritional, medical, religious and social uses which have been reported elsewhere (Faparunsi, 1966; Odeyemi, 1977; Ikenebomeh and Omayuli, 1988; Uzogara et al., 1990; Iheonu, 2000), to have increasingly enhanced the demand for this natural product. Palm wine is consumed for its nutritional effect because of its probiotic content (Heller, 2001; Lourens-Hattingh and Wiljoen, 2001; Ezeronye, 2004). Palm wine has been used locally as an extractant; solvent for extracting active ingredients from leaves, barks and stems of some medicinal trees used in the treatment of various ailments which include malaria, dental problems, yellow fever, pains and stomach disorders. It is also used locally in the treatment of skin rashes in children (Uraih et al., 1992).

Antimicrobial agents are substances which can be used to control (kill or inhibit) microbial growth and survival on or in a body or an environment (Mackie and McCartney, 1989). Many efforts have been made to discover new antimicrobial agents from various kinds of sources such as micro-organisms, animals, plants and plant products.

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One of such resources is folk medicine. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko et al., 2002).

The high cost of commercially produced antimicrobial agents coupled with increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to the antimicrobial agents raises the specter of untreatable bacterial infections and adds urgency to the search for new antimicrobial agents (Sieradzki *et al*., 1999). Hence the need for testing this local beverage for antimicrobial property, this project was therefore carried out to screen some of the locally produced alcoholic beverages for their antimicrobial properties using palm wine. To this effect, the antimicrobial action of palm wine was assayed on six organisms which are *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aureginosa, Bacillus species and a* fungus *Candida albicans* to ascertain the rationale for its use in traditional medicine.

MATERIALS AND METHODS

Collection of samples

The test organisms: *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aureginosa*, *Bacillus sp.* and *Candida albicans* were obtained from the clinical culture collections of the University of Ilorin Teaching Hospital, Ilorin, Nigeria. The organisms were maintained by sub culturing after every two weeks.

The test sample, palm wine was obtained directly from palm wine tappers from neighboring villages around Ilorin township at an agreed price to ensure originality. Methylated spirit was obtained from the Department of Biological Science Laboratory of the University of Ilorin, Ilorin, Nigeria.

Determination of the Physical Properties of Samples:

The pH was measured using a pH meter, Model 292, Mk_2 Pye Unicam; alcoholic content was measured with a hydrometer, Model PSL, UK while the temperature was taken with a thermometer.

The palm wine used was centrifuged using a bench centrifuge to obtain clear liquid which was sterilized in autoclave to rid of the resident organisms before they were tested against the organisms.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was carried out following the modified bacteria broth dilution method as described by the National Committee for Clinical Laboratory Standard (NCCLS). A discontinuous dilution of the test sample was made into peptone water to obtain various concentrations of the test samples ranging from 10% to 90% volume by volume of the test samples in peptone broth in test tubes. An undiluted sample was set up in each case (100% concentration). Each tube was inoculated with 0.5ml of 10^5 CFU/ml of a day old culture of the test organisms. Control tubes containing the undiluted samples were also set up in each case. The tubes were incubated at 37°C for 24 h for the bacteria and 48 h for the yeast. The lowest concentration of the test samples that did not allow any visible growth of the test organism in broth culture was regarded as the MIC in each case, (NCCLS, 1988; Collins et al., 1995).

Determination of Minimum Cidal Concentration (MCC)

After culturing the test organisms separately in the broth containing various concentrations of the test samples, the broth were inoculated onto freshly prepared agar plates to assay for the minimum cidal effect. The cultures were incubated at 37°C for 24 h. The lowest concentration of palm wine that does not yield any colony growth on the solid medium after the incubation period was regarded as MCC (Ellen et al., 1990; Alade and Irobi, 1993).

Agar- well diffusion method

Palm wine was tested for its antimicrobial action against bacteria and fungi using agar-well diffusion method following the procedures described by Biradar et al. (2007). The microorganisms were swabbed onto their respective agar. A sterile cork borer of diameter 5mm was used to make wells on the agar plates. 0.1ml of each of the test samples was dispensed in each well. The plates inoculated with *C. albicans* were kept at room temperature (25-27ºC) for 48 h while the other plates were incubated at 37^0C for 24h. Then, the zone of inhibition around each well was measured using a transparent metric ruler and recorded. Each test was conducted in triplicate and the average diameter was taken.

RESULTS

The physical characteristic of the palm wine is as shown in Table 1. It was whitish in color, pH 3.5, alcoholic content of 5.0 and temperature of 28^0C.

Palm wine was not able to inhibit the growth of *Bacillus sp* and *Candida albicans* but it displayed inhibitory action on the remaining organisms. It was noted that the

Table 1. Physical Characteristic of the Test Samples.

Physical characteristics of samples	Palm wine	Methylated spirit
Colour	whitish	Colourless
pH	3.5	6.5
Alcoholic content	5.0	70
Temperature	28	29

Table 2. Minimum Inhibitory Concentration (MIC) of Palm Wine on the Tested Organisms

Test organisms	Sterilized palm wine (%)	Methylated spirit (%)
Gram positive organisms		
Bacillus sp.	-	20
Staphylococcus aureus	40	20
Gram negative organisms		
Escherichia coli	50	10
Proteus mirabilis	20	10
Pseudomonas aureginosa	35	10
Fungi		
Candida albicans	-	10

-: no effect

Table 3. Minimum Cidal Concentration (MCC) of Palm Wine on the Tested Organisms**.**

Test organisms	Sterilized palm wine (%)	Methylated spirit (%)
Gram positive organisms		
Bacillus sp.	-	30
Staphylococcus aureus	Ud	30
Gram negative organisms		
Escherichia coli	Ud	20
Proteus mirabilis	30	20
Pseudomonas aureginosa	Ud	20
Fungi		
Candida albicans	-	20

-: no effect; Ud: undiluted sample

inhibitory action of palm wine on the organisms reduces as dilution increases. The minimum inhibitory concentrations are as shown on Table 2.

The minimum bactericidal concentration for most of the organisms tested was the undiluted sample. The diluted samples showed cidal effect only on *Proteus mirabilis*; one of the gram negative organisms. (Table 3).

The result of the antimicrobial activity of palm wine on the tested organisms through agar well diffusion method was as shown in Table 4. Palm wine was able to inhibit the growth of the organisms except *Bacillus* sp and *Candida albicans*.

DISCUSSION

Palm wine was found to exhibit bactericidal effect on all the organisms except on *Bacillus sp* and *Candida albicans.* This may be due to the fact that *Bacillus* is *a* known spore former and spores are known to be more resistant to antimicrobial agents than vegetative cells add reference. It has also been reported that *Candida albicans* is one of the resident organisms in palm wine and this may account for its ability to survive in palm wine. (Elijah et al., 2010) also Akinyanju and Oloruntoba, (1986) reported that gram positive organisms survive better than the gram negatives in palm wine and that the antimicrobial action of palm wine against *E. coli*, a gram negative coliform can be attributed to a heat labile factor which it contains.

The bacteristatic and bactericidal activities of palm wine reduced drastically on dilution this shows that higher concentrations are needed for effective action. Akinsanya, (1980) reported that when a bacteristatic

agent is excessively diluted, it becomes in activated and bacteria may even survive in it also when a bactericidal agent is excessively diluted it becomes bacteristatic. The fact that these organisms needs higher concentrations of palm wine to inhibit or kill them may also be due to their cell wall composition. According to the report of Shanson, 1990, the action of alcoholic agents on microorganisms consist in denaturation of protein and lyses of cell membrane. *Pseudomonas aureginosa* has also been noted to exhibit high resistance against many antimicrobial agents as a result of either their ability to metabolize the antimicrobial agents or due to their cell wall composition (Nakahara and Kozukue, 1982).

The ability of *Staphylococcus aureus* to produce an extracellular substance which makes it less susceptible to antimicrobial agents has also been reported by earlier author, (Saxena et al., 1983). The antimicrobial action of palm wine on these organisms agrees with the findings of earlier authors who has used it in various ways as extractant (solvent) for different parts of medicinal plants used in the local treatments of various diseases of microbial origin, Aibinu,*et al.,* 2007, Odunayo *etal,.* 2007 and Ikenebomeh and Omayuli, (1988). The findings in this work showed palm wine to be active against four of the tested organisms including *S. aureus and P. aureginosa* which are among bacteria that are now showing resistance to most antiseptic agents and antibiotics Gislene *et al.,(*2000) and Sieradzki, (1999).

CONCLUSION

Palm wine could be used as an extractant for local medicinal herbs in traditional medicine to enhance or increase the effectiveness of these herbs against the organisms on which they are specifically active. This research work has open up doors for more work to be done on discovering the mode of action of palm wine and the active ingredients in it. This could possibly be synthesized and incorporated into synthetic drugs to treat infectious diseases of microbial origin that are particularly susceptible to them.

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