Preservative Potential of *Zingiber officinale, Thaumatococcus daniellii* and Ver*nonia amygdalina* in *Raphia hookeri* and *Elaeis guineensis* Palm wine

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Abstract
Palm wine is of high economic and nutritional importance and plays a significant role in social and cultural practices in Africa, it is often recommended to infants whose mother cannot produce the necessary milk for the nursing, but one major problem still faced by the palm wine consuming public is preservation, which limits its distribution. Therefore, this study was aimed to determine the Preservative potential of Plant leave such as Zingiber officinale, Thaumatococcus danielli and Vernonia amygdalina on the shelf life of Raphia hookeri and Elaeis guineensis Palm wine. Freshly tapped palm wine from Raphia raphia (raphia palm tree) and Elaeis guineensis (oil palm tree) were collected at the point of tapping from traditional palm wine tapper in Choba, Rivers State, The plant leaves and rhizome were obtained from Mile 3 market and transported wrapped in dry cellulose papers. The plant materials were authenticated at Department of crop science, Rivers State University science. On arrival at the laboratory the plant materials were picked and washed with sterilized water and absolute alcohol respectively. Microbiological analysis was carried out on the palm wines using standard procedure. The preservative potential test was done using standard methods. an aliquot (0.1ml) from each of the concentrations were then plated on pre-dried respective media after 24hour, inoculation and spreading continues in 48, 72 96, and 120 hours respectively After incubation, the colonies on the plates were counted and converted to Logarithm base ten (log_{10}). Preservative effect was measured using the total viable count (TVC) as a directory. Results of total heterotrophic bacterial, fungal counts obtained from two the different fresh palm wine ranges from 1.58 x 10^4 cfu/ml to 2.67 x 10^4 cfu/ml for the total heterotrophic bacterial count while total heterotrophic fungal count ranges from 1.60 x 10^3 cfu/ml to 1.64 x 10^3 cfu/ml. Results of effect of the various plant Leaves on pH showed that Palm wine without plant leaves ranges from 1.99 (120 Hour) to 6.75 (24 hour), Palm wine preserved with Ginger (Z.officinale) ranges from 3.56 (120 Hour) to 6.30 (24 hour) Palm wine preserved with Moimoi leaf (T.danieli) ranges from 3. 09 (120 Hour) to 5.60 (24 hour) Palm wine preserved with Bitter leaf (Vernonia amygdolina) ranges from 2.69 (120Hour) to 6.75 (24 hour). The results of preservative effect of the plant leaves and rhizome shows that statistically, there was a significant difference of the effect of concentrations of the respective plant leaves and rhizome at 0.05 confident limit across the two palm wine used in this study especially the synergetic effect. The percentage survival of the total heterotrophic fungi and bacteria in palm wine decreases with increased concentrations and time, the total heterotrophic bacteria decreased rapidly than the fungal isolates. This study have shown that the preservative problem of palm wine can be solved with the use of natural plant leaves.

Keywords: *Preservation, plant leaves, rhizome and percentage mortality*

INTRODUCTION

Palm wine is a rich nutrient medium containing sugars, proteins, amino acids, alcohols and minerals (Ukhun *et al.*, 2005). It contains a lot of water soluble vitamins. Ibegbulem *et al.* (2013) reported that it is a good source of vitamins B1 (thiamine) and C (ascorbic acid). According to the authors, palm wine yeast, *Saccharomyces cerevisiae*, is able to concentrate large quantities of thiamine, nicotinic acid and biotin and thus form enriched products. West African palm wine is particularly rich in vitamins B12, which is very important for people with low meat intake and those who subsist primarily on vegetarian diets (Ezeagu and Fafunso, 2003; Palm wine is generally referred to as a group of alcoholic beverages obtained by fermentation of the saps of palm trees (Amadi *et al.*, 2016). It is consumed throughout the tropics and appears as a whitish liquid produced by natural fermentation of the sap of *Elaeis guineensis* and *Raphia hooker*i. The unfermented sap is clean, sweet, colourless syrup containing about 10 - 12% sugar, which is mainly sucrose. It is a refreshing beverage widely consumed in south eastern Nigeria and other parts of the world particularly Asia and Southern America. Although palm wine may be presented in a variety of flavours, ranging from sweet (unfermented) to sour (fermented) and vinegary, but is mostly enjoyed by people when sweet (Elijah *et al.*, 2010).

Palm wine is of high economic and nutritional importance and plays a significant role in social and cultural practices in Africa. As a result of the nutritional value of the unfermented sap, it is often recommended to infants whose mother cannot produce the necessary milk for the nursing (Ezeagu and Fafunso, 2003). Ibegbulem *et al.* (2013) reported that as a good source of vitamins B1 (thiamine) and C (ascorbic acid), palm wine offers supplemental nutrition to a meal. The drink is a rich nutrient medium containing sugars, proteins, amino acids, alcohols and minerals (Ukhun *et al.*, 2005).

The unfermented palm sap is a sweet, transparent juice with a sugar content of 100 -144 g/kg, a pH of 7.0 - 7.4 and traces of ethanol (Lasekan *et al.*, 2007), while the fermented sap, is whitish and has a pH of about 3.6 and alcohol content of 3.3 - 4.0%, depending on the stage of fermentation at which the wine is consumed (Lasekan and Abbas, 2010). Thus palm wine is consumed for various reasons. It may be consumed for its thirst quenching effect, sweetness or stimulating effect. One major problem still faced by the palm wine consuming public is preservation, which limits its distribution. It is therefore important that palm wine is preserved in its natural state. However, palm wine contains a heavy suspension of live yeast and bacteria (Okafor, 2006). These microorganisms metabolize the sugars thus removing the pleasant sugary taste of the fresh wine within 36 – 48 hours of production, and produce various organic acids (Faparusi and Bassir, 2016 ; Bassi, 2002).

Although attempts have been made towards the preservation and shelf-life extension of palm wine through bottling, use of chemical additives and addition of plant extract have greatly affected the organoleptic quality of the product because palm wine has a significant role in several nutritional, medical, religious and social uses such as traditional wedding ceremonies, traditional religious ceremonies or festivals, prayers and it is good for malaria (Olasupo and Obayori, 2003; Chandrasekhar *et al.*, 2012).

But the rapid deterioration in organoleptic quality of palm exudates by the natural flora of the fermenting sap constitutes the major problem facing palm wine production and distribution. This problem became eminent ever since palm wine consumption became popular and has been a subject of intense study, therefore this study was aimed to determine the Preservative Potential of zingiber officinale, *Thaumatococcus daniellii* and *vernonia amygdalina* in *Raphia hookeri* and *Elaeis guineensis* Palm wine. *Thaumatococcus daniellii* is a plant species from Africa, known for being the natural source of thaumatin, an intensely sweet protein which is of interest in the development of sweeteners. When the fleshy part of the fruit is eaten, this molecule binds to the tongue's taste buds, causing sour foods to taste sweet

(Jirovetz *et al.*, 2001). Ginger (*Zingiber officinale*) is a flowering plant whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine, *Vernonia amygdalina*, a member of the daisy family, is a small shrub that grows in tropical Africa. *V. amygdalina* typically grows to a height of 2–5 m (6.6–16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. Its bark is rough (Ijeh and Ejike 2011).

MATERIALS AND METHOD

2.1. Study Area

The study was carried out in Choba of Obio-Akpor local government area in the metropolis of Port Harcourt, one of the major centres of economic activities in Nigeria, and one of the major cities of the Niger Delta, located in Rivers State.

Freshly tapped palm wine from *Raphia raphia* (raphia palm tree) and *Elaeis guineensis* (oil palm tree) were collected at the point of tapping from traditional palm wine tapper in Choba, Rivers State, Nigeria. The samples were collected and transported to the laboratory in coolers equipped with ice packs within one hour of tapping in 1.5 litres sterile flasks for treatments and analyses. This procedure is intended to keep the samples at about 2 - 4°C. (Ibekwe *et al.* 2006).

2.2.2: Plant Leaves and Rhizome

The plant preservatives used were Moimoi leaf (*T.danielii*), Ginger (*Z.officinale*) and bitter leaf (*Vernonia amygdalina*). They were obtained from Mile 3 market. The leaves were transported wrapped in dry cellulose papers. The plant materials were authenticated at Department of crop science, Rivers State University science. On arrival at the laboratory the plant leaves were picked and washed with sterilized water and absolute alcohol respectively. The ethanol was subsequently rinsed with the sterilized distilled water and blended to fine power after drying before used.

2.3: Determination of Microbial Load

Determination of microbial load of the samples was done based on the method described by Rashed *et al.*, (2013). Serial dilutions of samples were made up to 10^{-3} with sterile normal saline. Exactly 0.1ml of each dilution was evenly spread on nutrient agar medium and incubated at 37^{o}C for 24 hours. Plates were screened for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated as colony forming unit per ml (cfu/ml). The load of specific microorganisms was determined by plating on selective media. Nutrient (NA) and Sabroud dextrose agar (SDA) medium, respectively. In each case plating was done in duplicates and counts taken from plates that had less than 300 colonies. Estimation of bacteria load was performed by standard method according to International Commission on Microbiology Specification for Food (1998).

2.3: Determination of Preservative Potential of Plant Leaves

The Preservative potential of plant leaves were determined by setting up fifteen sterile plastic bottles. The test was carried out in five (5) separate sterile plastic bottles containing one hundred milliliter of palm wine. In each of the plastic bottles, the four different concentrations (%); 2.5, 5, 10, and 25 of the stock toxicant) were added separately.

These concentrations were obtained by aseptically transferring 2.5, 5, 10, and 25 grammes of the dried blended plant leaf in to 100ml of palm wine respectively (Nrior and Kpormon 2018). The control contains only the palm wine.

Aliquot (0.1ml) from each of the concentrations of the suspension of palm wine and plant leaf was then plated out using spread plate technique on respective media (Nutrient agar and Sabouraud Dextrose agar) at constant interval of 24 hour, as follows: 24, 48, 72, 96 and 120

hours respectively and was incubated for 24 hours at 37± 2ºC exception of the SDA plates that were incubated for 72 at 28ºC. After incubation the total viable count on the plate were taken and converted to $\log_{10}$ (Kpormon *et al.,* 2018). This process was repeated for the three plant leaves in the two types of palm wine.

2.4. Determination of Percentage log survival of the test organisms

The percentage log survival of the Total heterotrophic bacteria and fungi were determined by dividing logarithmic count of the toxicant concentration, by the logarithmic count of the control and multiplying by 100.

Percentage (%) logarithmic survival

$$= \frac{\text{Log } \mathbf{C}}{\text{Log c}} \times 100$$

Where;

Log **C** = logarithmic count of the toxicant, Log c = logarithmic count of the control

2.5 Percentage log mortality

The Percentage (%) log mortality of the Total heterotrophic bacteria and fungi exposed to the toxicant were determined by subtracting the one hundred from the value of the percentage log survival

Percentage (%) = 100 - % log survival

3.6: pH Determination

Change in pH of fermenting palm wines containing amended with the respective plant leaves, was measured using the pH meter (Electronic Instrument, Model 7060, England). Results were obtained in triplicate. These were done on day one at and continue after 24, 48, 72, 96 and 120 hours respectively

2.7: Statistical Analysis

The data obtained during the study was analyzed statistically using a computer based program, SPSS version 22 for analysis of variance (ANOVA) of the data in the respective ecosystems.

3.0 RESULTS AND DISCUSSION

The percentage survival of the total heterotrophic fungi and bacteria in palm wine decreases with increased concentrations and time, the total heterotrophic bacteria decreased rapidly than the fungal isolates. The organisms were exposed to various concentrations (%); 0, 2.5, 5. 10, and 25 at a constant interval of 24 hours for 120 hours. The results obtained were statistically analyzed and are presented in Tables 1 to 4 respectively. Table 1 shows singly and synergetic effect of the various leaves on total heterotrophic fungi obtained in *Raphia hookeri* palm tree with respect to time and concentration in percentage. Table 2 shows singly and synergetic effect of the various leaves on total heterotrophic bacteria obtained in *Raphia hookeri* palm tree with respect to time and concentration in percentage. Table 3 shows singly and synergetic effect of the various leaves on total heterotrophic bacteria obtained in *Elaeis guineensis* palm tree with respect to time and concentration in percentage. Table 4 shows singly and synergetic effect of the various leaves on total heterotrophic fungi obtained in *Elaeis guineensis* palm tree with respect to time and concentration in percentage.

The results also shown that statistically, there was a significant difference of the effect of concentrations of the respective plant leaves and rhizome at 0.05 confident limit across the two palm wine used in this study especially the synergetic effect.

Table 4.3: Effect of Plant Leaves and rhizome on the Percentage Survival Total heterotrophic Fungi in Raphia *hookeri Palm wine*

Concentration (%)	Moimoi leaf	Bitter leaf	Ginger	M+B+G
Control	100.00±0.00 ^{d}	100.00±0.00 ^{d}	100.00±0.00 ^{d}	100.00±0.00 ^{b}
2.5	97.18±2.17 ^{c}	98.12±0.56 ^{c}	97.10±1.06 ^{c}	91.06±4.97 ^{a}
5	95.88±1.80^{bc}	96.62±0.52 ^{b}	95.84±0.84 ^{bc}	89.96±5.09 ^{a}
10	94.82±1.88 ^{ab}	95.86±0.83 ^{ab}	93.84±1.59 ^{ab}	89.18±0.33 ^{a}
25	92.92±0.80 ^{a}	94.52±1.98 ^{a}	91.76±3.10 ^{a}	88.34±5.44 ^{a}

Table 4.4: Effect of Plant leaves and rhizome on the Percentage Survival Total heterotrophic Bacteria in *Raphi ahooker*

Concentration (%)	Moimoi leaf	Bitter leaf	Ginger	M+B+G
control	100.00±0.00^{c}	100.00±0.00 ^{d}	100.00±0.00 ^{c}	100.00±0.00 ^{c}
2.5	94.10±3.28^{b}	93.88±2.27 ^{c}	93.08±3.79 ^{b}	92.22±2.22 ^{b}
5	92.42±3.26 ^{ab}	90.56±1.37 ^{b}	91.38±3.17 ^{ab}	89.48±1.76 ^{b}
10	90.600±3.73 ^{ab}	88.70±2.09 ^{ab}	88.98±3.79 ^{ab}	86.98±3.84 ^{ab}
25	89.40±4.30 ^{a}	87.42±1.82 ^{a}	87.22±4.46 ^{a}	81.90±7.38 ^{a}

Table 4.5: Effect of Plant leaves and rhizome on the Percentage Survival Total heterotrophic Bacteria in *Elaeis guineensis*

Concentration (%)	Moimoi leaf	Bitter leaf	Ginger	M+B+G
Control	100.00±0.00^{c}	100.00±0.00 ^{c}	100.00±0.00 ^{d}	100.00±0.00 ^{c}
2.5	95.02±2.83^{b}	92.80±2.19 ^{b}	92.86±2.40 ^{c}	91.72±2.11 ^{b}
5	93.18±3.33^{ab}	92.14±2.27 ^{b}	89.86±1.57 ^{b}	88.76±1.65 ^{ab}
10	91.46±2.10 ^{a}	90.18±2.36 ^{ab}	87.76±2.82 ^{ab}	86.86±4.05 ^{a}
25	90.04±2.29 ^{a}	88.78±2.27 ^{a}	86.28±3.02 ^{a}	85.24±4.32 ^{a}

Table 4.6: Effect of Plant leaves and rhizome of Total heterotrophic Fungi in *Elaeis guineensis*

Concentration (%)	Moimoi leaf	Bitter leaf	Ginger	M+B+G
control	100.00±0.00 ^{d}	100.00±0.00 ^{e}	100.00±0.00 ^{c}	100.00±0.00 ^{d}
2.5	98.54±1.06 ^{cd}	98.24±0.64 ^{d}	96.26±1.83 ^{b}	94.50±1.01 ^{bc}
5	97.46±1.31 ^{bc}	97.20±.30 ^{c}	95.06±1.86 ^{ab}	93.20±1.09 ^{b}
10	96.48±1.30^{ab}	96.18±0.62 ^{b}	94.16±2.04 ^{ab}	92.00±1.24 ^{b}
25	95.26±2.21 ^{a}	94.60±1.42 ^{a}	93.38±2.64 ^{a}	88.70±2.47 ^{a}

The result obtained in the present study revealed that combination of the plant had more preservative potential of the palm wine compared to single strength of the individual plant leaves. Some of the constituent of plant leaves and rhizome used contains chemical that have ability to inhibition or kill microorganisms, reduce the metabolic and enzymatic reactions of the fermenting microorganisms in the wines, could be attributed to the increase of percentage mortality with increasing concentration and time obtained in the treated palm wine compared to the control. The greatest obstacle to the distribution of palm wine is the fact that once

fermentation starts, it usually continues , this plant product delayed souring of the wine (Okafor, 2005), and lowered its titratable acidity (Ojimelukwe, 2000).

Effect on of Plants Leaves and rhizome on the Hydrogen Concentration in Palm wine during storage (pH)

Figures 1 to 3 shows the effect of preservation of palm wine with different plant leaves and rhizome on the pH values of fermenting palm sap during a 120 hours of storage period at room temperature (29 - 32 °C). There was a decrease in the pH values of all samples as the storage period increased with time. A gradual decrease in the pH values was observed in the treated palm wine while the untreated which served as control decrease rapidly as time increases, these indicates that the plant leaves reduced the metabolic activities of the fermenting microbe present in the palm wine (Abiose and Adedeji, 1994). Owuama and Saunders (1990) reported that palm wine fermentation is a yeast/lactic acid bacteria fermentation. Decrease in the pH values as the storage period increased was higher for palm wine which was not treated with plants leaves. As the concentration of the respective plant leaves used to preserve the palm sap increased, the changes in pH values became less. Similar observations have been reported in palm wine treated with *S. gabonensis* (Ojimelukwe, 2000).

The concentration of Plant leaves and rhizome significantly affected ($P < 0.05$) the pH values of the fermenting palm sap.

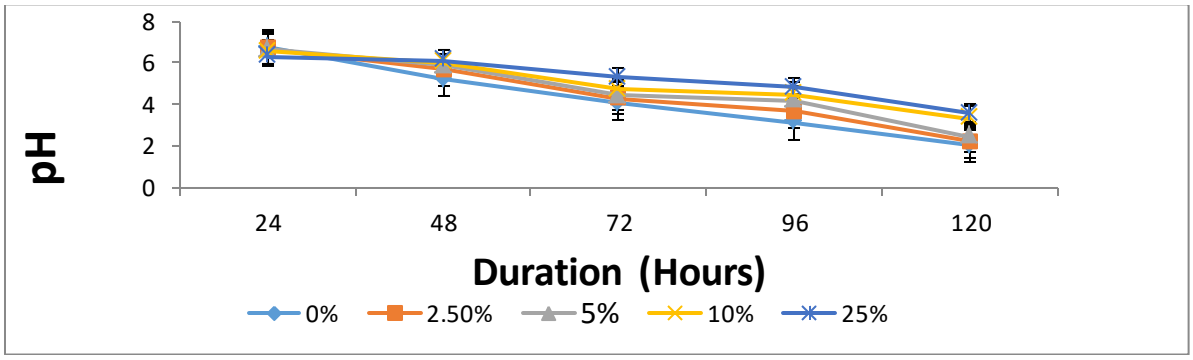

Figure 4.4: Effect of Ginger (*Z.officinale*) pH of Palm wine After 120 hours of Storage at Room Temperature

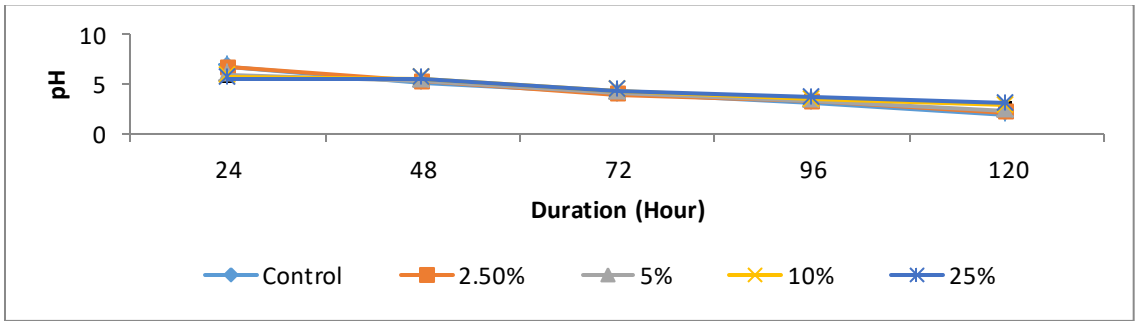

Figure 2: Effect of Moimoi leaf (*T.danielii*) pH of Palm wine After 120 hours of Storage at Room Temperature

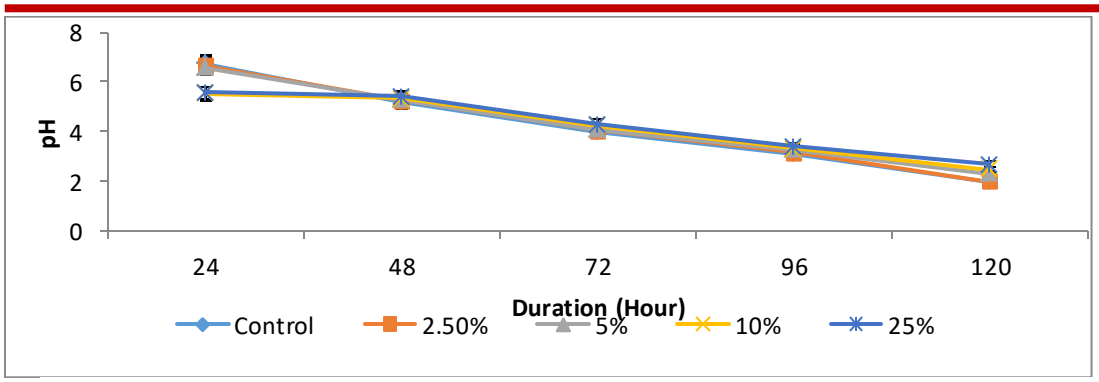

Figure 3: Effect of Bitter leaf (*Vernonia amygdolina*) pH of Palm wine After 120 hours of Storage at Room Temperature

Conclusion

The findings from this study showed that statistically, there were significant difference of the effect of various concentrations of the respective plant leaves and rhizome at 0.05 confident limit across the two palm wine used in this study especially the synergetic effect. Therefore Combination of ginger rhizome, Bitter and Moimoi leaves is recommended for natural preservation of palm wine.

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