

# Synergistic Bio-preservative Effects of *Vernonia Amygdalina* Leaves and *Sacoglottis Gabonensis* Stem Bark on Palm Wine from *Elaeis Guineensis* and *Raphia Hookeri* from Uturu, Nigeria

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**Abstract** The synergistic bio-preservative effects of leaves and stem bark of *Vernonia amygdalina* and *Sacoglottis gabonensis* respectively on two (2) palm wine types namely, *Elaeis guineensis* and *Raphia hookeri* from Uturu-Nigeria was evaluated. The microbiological and biochemical changes of the palm wine brands were determined. *R. hookeri* brands were found to support more heterotrophic and coliform populations than the *E. guineensis*, while the later contained more yeast species. Identification of isolated species revealed the presence of *Bacillus* sp., *Micrococcus* sp., *Lactobacillus* sp., *Brevibacterium* sp. and *Saccharomyces* sp. from *E. guineensis* and *R. hookeri*. Moreso, heterotrophic counts and pH were observed to decrease as the fermentation time progressed. The combination of both *V. amygdalina* and *S. gabonensis* as preservatives lowered the bacterial and fungal load compared to the control and individual plant preservatives and reduced the rate of CO<sub>2</sub> emission as well as keeping the pH fairly constant.

**Keywords:** synergistic, bio-preservative, *Vernonia amygdalina*, *Sacoglottis gabonensis*, palm wine, Uturu

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## 1. Introduction

Palm wine is the fermented sap of various palm trees especially Palmyra, silver date palm and coconut palms. Palm wine can be obtained from the young inflorescence either male (or) female ones. Palm wine is an alcoholic beverage that is made by fermenting the sugary sap from various palm plants. It is collected by tapping the top of the trunk by felling the palm tree and boring a hole into the trunk. It is a cloudy whitish beverage with a sweet alcoholic taste and a very short shelf life of only one day. The wine is consumed in a variety of flavors, varying from sweet-unfermented to sour-fermented and vinegary. There are many types of the palm wine products and is particularly common in parts of Africa, South India and Mexico. Some of the local names for the product include Emu and Ogororo in Nigeria and Nsafufuo in Ghana [1].

The wine is an excellent substrate for microbial growth and fermentation starts soon after the sap is collected and within an hour or two becomes reasonably high in alcohol (up to 4%); if allowed to continue to ferment for more than a day, it starts turning into vinegar [1].

Palm wine is consumed throughout the tropics and appears as a whitish liquid produced by natural

fermentation of the sap of *Elaeis guineensis* and *Raphia hookeri* [2]. The unfermented sap is a clean, sweet, colourless syrup containing about 10 - 12% sugar, which is mainly sucrose [3]. Upon fermentation by the natural microbial flora, the sugar level decreases rapidly as it is converted to alcohol and other products [4] whereas, the sap becomes milky-white due to the increased microbial suspension resulting from the prolific growth of the fermenting organisms [5].

*Vernonia amygdalina* (Del.) commonly called bitter leaf is the most widely cultivated species of the genus *Vernonia* which has about 1,000 species of shrubs [6]. It belongs to the family Asteraceae. It is popular in most West African countries including Nigeria, Cameroon, Gabon and Congo Democratic Republic.



Figure 1. *Vernonia amygdalina* leaves

*Sacoglottis gabonensis* is a tropical rainforest tree found in the tropical rainforest region of Africa and America. It belongs to the family Humiriaceae. In certain rural communities of Nigeria especially Abia, Akwa-Ibom, Rivers, Delta, Edo and Imo State, the stem bark of this tree is commonly used as an additive to palm wine [7].



Figure 2. *Sacoglottis gabonensis* leaves and stem

This study is therefore aimed at ascertaining microbial and biochemical changes associated with non-preserved and the combined bio-preservative effect of *V. amygdalina* and *S. gabonensis* on the two brands of palm wine.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Sample Collection

Fresh palm wine samples from oil palm tree (*E. guineensis*) and Raphia palm (*R. hookeri*) was separately collected from traditional palm wine collectors from Uturu, Abia State, Nigeria. The freshly tapped samples were collected using a total of 10 pre-sterilized 100 ml capacity bottles with screw caps. The perforated screw caps were plugged with sterile non-absorbent cotton wool. Each of the two different palm wine types were collected in 5, 100 ml bottles. The samples were transported to the laboratory in a cooler equipped with packs of freezing mixture of salt and ice-block for analysis within 1 h of collection; this was to help reduce the fermentation rate.

#### 2.1.2. Plant Materials

Leaves and stem barks of *Vernonia amygdalina* and *Sacoglottis gabonensis* respectively were collected from National Research Institute Umudike, Abia State, Nigeria. The plant materials were authenticated by the Department of Plant Science, Abia State University Uturu, Nigeria. The plant materials were washed with sterilized water and then with absolute ethanol and shade dried ( $25^{\circ}\text{C} \pm 2$  for 14 days). The dried leaves and stem bark were then ground to a fine powder in a mechanical blender and mixed together.

## 2.2. Methods

### 2.2.1. Treatments

A total of 8 bottles, 4 for each of the two palm wine samples, were labeled thus:

- Control (C):** Sample without preservative
- T0:** Sample with only *Vernonia amygdalina*
- T1:** Sample with only *Sacoglottis gabonensis*
- T2:** Sample with both Plant materials.

### 2.2.2. Preservation Treatments

Three – 60 ml sample of each Raphia and Oil Palm wine were treated with a total of 3 types of preservatives namely, *Vernonia amygdalina* only (T<sub>0</sub>), *Sacoglottis gabonensis* only (T<sub>1</sub>), combination of *Sacoglottis gabonensis* and *Vernonia amygdalina* (T<sub>2</sub>); whereas, the one sample bottle was left without any form of preservative (C) as control. The treatment was carried out by adding 10 mg of each powdered traditional preservative to the sterile sample bottles, but 5 mg each of both plant materials for T<sub>2</sub>. Thereafter, 60 ml of fresh palm wine sap were added, gently shaken to mix and allowed to stand in a laboratory glass cabin sterilized using 2.5% acid alcohol.

### 2.2.3. Microbial Isolation

1ml aliquots of each palm wine sample were taken aseptically at 24, 48, 72, 96 and 120 hours of fermentation and were serially diluted 10-fold in 0.1% (w/v) peptone. 1ml dilutions were plated out in duplicates using spread plate method [8], on tryptone soy agar (Oxoid) for total heterotrophic bacterial count, MacConkey agar (Oxoid) for the total coliform count and Sabouraud dextrose agar (Oxoid) containing 0.05 mg/ml chloramphenicol for yeast count as described by [9]. The inoculated plates were incubated aseptically at 30°C for 24 h for bacteria and 24–48 h for the yeast. Acceptable plated were those that contained between 30-300 cfu/ml. They were stored on agar slants at 40°C for characterization.

### 2.2.4. Characterization of Isolates

The Isolates were grouped according to their colonial morphology and cell characteristics. The colonies were counted and re-isolated in pure culture using the medium on which they had grown as described by [10]. Isolates were thereafter subjected to biochemical tests as described by [11]. The probable identities of the isolates were determined as recommended by [12].

### 2.2.5. Chemical Analysis

The method described by [13] was adopted to determine the rate of CO<sub>2</sub> evolution and pH of the sample.

### 2.2.6. Data Analysis

The experimental results were expressed as mean  $\pm$  standard error of mean (SEM) of three replicates. The results were processed using Microsoft Excel 2007 and Origin 6.0.

## 3. Results

The microbiological assays revealed that more total heterotrophic bacteria and coliform counts were obtained from palm wine from *R. hookeri* than *E. guineensis* while the later had more yeast counts than the former (Figure 3 to Figure 8). The mean occurrence of the bacterial genera and yeast revealed a sharp increase from 0–24 h for the total heterotrophic bacteria for both samples and gradually reduced as fermentation time increased in both test and control samples, while coliform counts gradually increased up to 72 h and started reducing in both samples. Yeast population showed a steady increase from 24 h of

fermentation to the 48 h. Thereafter, a sharp progressive decrease was observed from 72 h.

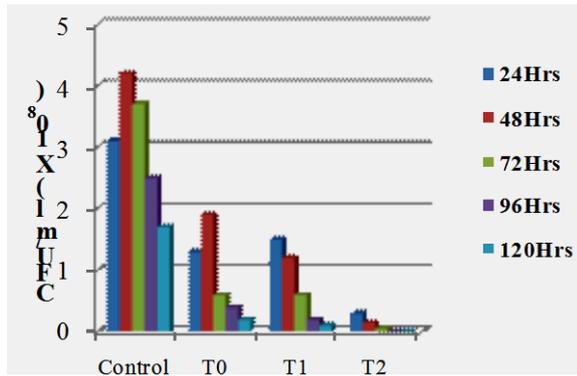


Figure 3. Total Heterotrophic counts of isolates in palm wine from *Elaeis guineensis*

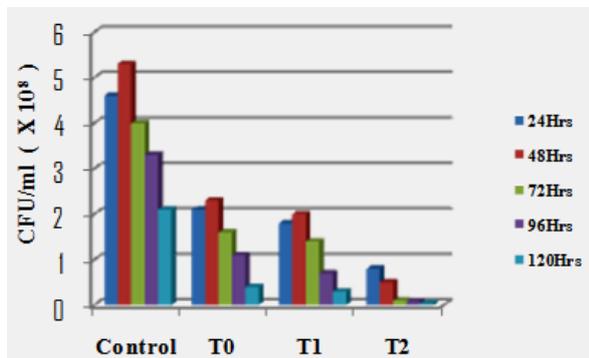


Figure 4. Total Heterotrophic counts of isolates in palm wine from *Raphia hookeri*

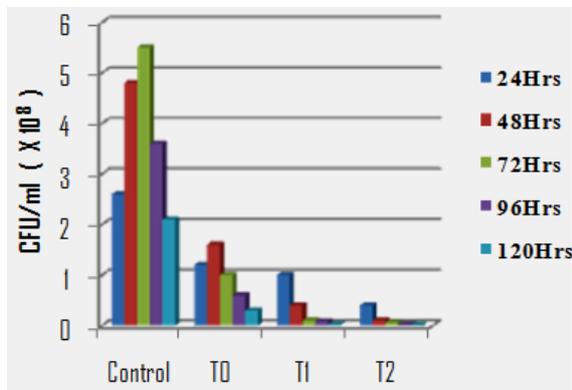


Figure 5. Total Coliform counts of isolates in palm wine from *Elaeis guineensis*

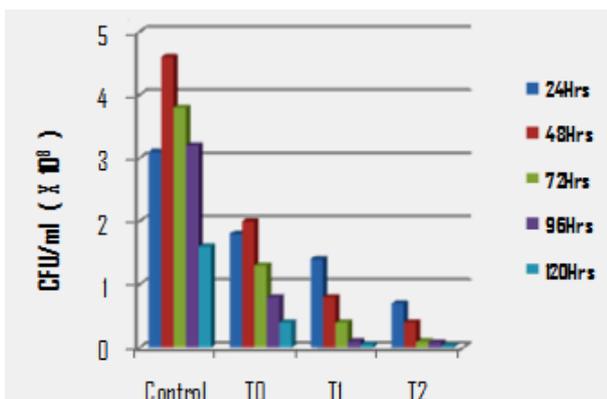


Figure 6. Total Coliform counts of isolates in palm wine from *Raphia hookeri*

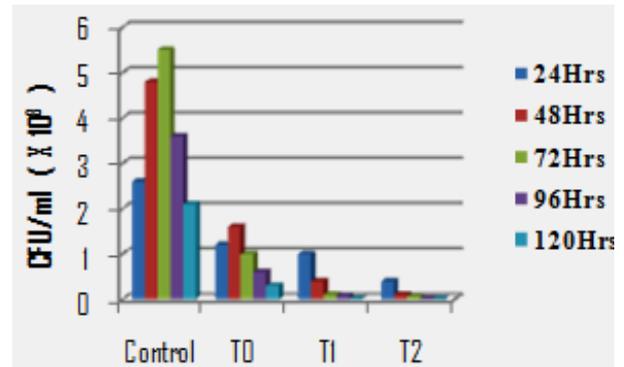


Figure 7. Total Yeast counts of isolates in palm wine from *Elaeis guineensis*

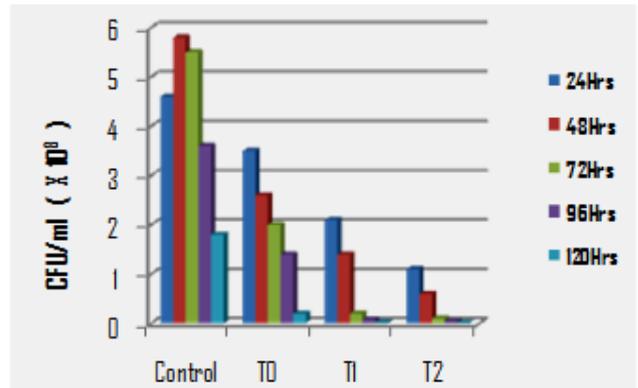


Figure 8. Total Yeast counts of isolates in palm wine from *Raphia hookeri*

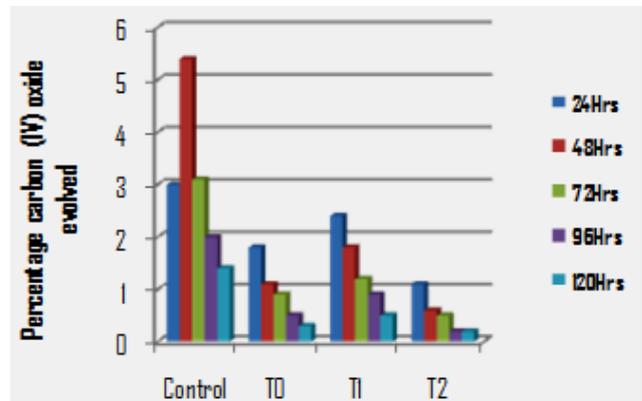


Figure 9. Percentage CO<sub>2</sub> released from palm wine harvested from *Elaeis guineensis*

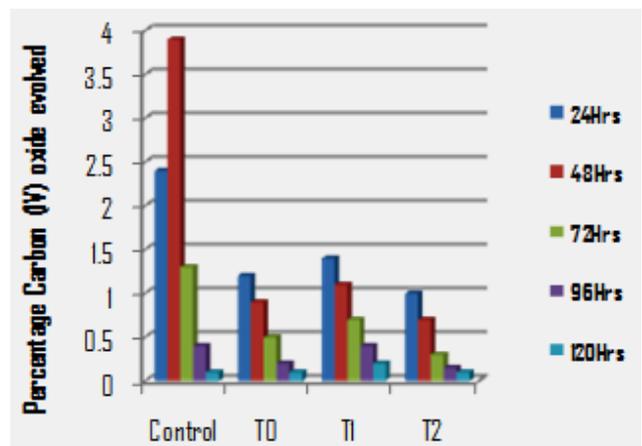


Figure 10. Percentage CO<sub>2</sub> released from palm wine harvested from *Raphia hookeri*

Generally, in all samples of *E. guineensis* and *R. hookeri*, low heterotrophic, coliform and yeast counts were observed to be far lower in the samples with both *V. amygdalina* and *S. gabonensis* compared to individual plants when used as preservatives.

The percentage carbon (iv) oxide (CO<sub>2</sub>) evolved during the different fermentation times are shown in Figure 9 and Figure 100 for palm wine from *E. Guineensis* and *R. hookeri* respectively.

The two types of palm wine samples showed considerable decrease in pH values as fermentation time increased in all samples except for the samples containing both *V. amygdalina* and *S. gabonensis* as shown in Table 1 and Table 2.

**Table 1. Effect of Single and Combined Plant Preservatives on pH of Palm Wine from *E. guineensis***

Duration of Fermentation (Hrs)	pH			
	C <sub>0</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>
24	5.50	5.80	6.00	6.40
48	4.20	5.20	5.60	6.10
72	3.60	4.60	4.90	5.90
96	2.60	4.20	4.50	5.50
120	2.20	3.80	3.60	5.20

**Table 2. Effect of Single and Combined Plant Preservatives on pH of Palm Wine from *R. hookeri***

Duration of Fermentation (Hrs)	pH			
	C <sub>0</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>
24	5.10	6.00	5.70	6.34
48	4.00	5.40	5.30	6.00
72	3.60	4.80	4.90	5.80
96	3.00	4.60	4.60	5.50
120	2.20	3.90	4.20	5.10

Isolation and identification test revealed the presence of more bacteria in the palm wine from *R. hookeri* than in *E. guineensis* palm wine. Isolates from *E. guineensis* include *Saccharomyces* sp., *Lactobacillus* sp., *Bacillus* sp. and *Brevibacterium* sp. Isolates from *R. hookeri* included all the isolates from *E. guineensis* except for *Micrococcus* sp. The survival rates of these isolates were affected by increase in fermentation time (Table 3).

**Table 3. Effects of Fermentation Time (Hrs) on the Survival Rate of Fungal and Bacterial species from Palm Wine**

Isolate	Fermentation/Isolation Time (Hrs)				
	24	48	72	96	120
<i>Bacillus</i> sp.	+	+	+	+	-
<i>Micrococcus</i> sp.	+	+	-	-	-
<i>Lactobacillus</i> sp.	+	+	+	+	-
<i>Brevibacterium</i> sp.	+	+	-	-	-
<i>Saccharomyces</i> sp.	+	+	+	-	-

## 4. Discussion

The total heterotrophic bacterial counts were relatively low in palm wine samples treated with *V. amygdalina* and *S. gabonensis* compared to those preserved with only one plant preservative and sample with no preservative at all. Peak heterotrophic bacterial counts were obtained after 24 hours fermentation except for the sample with no form of preservative. This agrees with [14] that observed higher bacterial counts after 24 hours fermentation of palm wine.

A gradual loss of bacterial and fungal viability was noticed as fermentation time increased from 48 hours to 120 hours. Five probable microbial isolates were identified; they include *Bacillus* sp., *Micrococcus* sp., *Lactobacillus* sp., *Brevibacterium* sp. and *Saccharomyces*

sp. The isolation and identification of these microbial species from fermenting palm wine is in line with the work of [15] that shows the presence of different microbial flora of exposed fermenting palm wine.

The isolation of *Micrococcus* sp. from fermenting palm wine possess health implications which might have been due to the exposure of freshly tapped wine; which supports the research done by [16], that showed various forms of pathogenic bacteria associated with exposed palm wine. The frequent Gastro-intestinal problems associated with drinking palm wine well over 24 hours could be attributed to the presence of pathogenic bacteria in palm wine.

The slow reduction in bacterial and fungal isolates from palm wine over the fermenting period could be attributed to the gradual depletion of fermenting sugar, production of organic acids and consequent reduction of pH.

The gradual reduction in viability of microbial isolates in palm wine preserved with plant materials could also be attributed to the presence of bioactive components present in the *V. amygdalina* and *S. gabonensis*. This supports the work of [17], which showed antibacterial effects of medicinal plants against pathogens.

However, [18] reported that preservation of palm wine could be achieved through inactivation of microorganisms at about 15 hours after tapping. The Combination of such methods with subsequent preservation using plant preservatives such as *V. amygdalina* and *S. gabonensis* could however extend the shelf life of palm wine even more.

## 5. Conclusion

This study therefore showed that combined use of *V. amygdalina* leaves and *S. gabonensis* stem bark may have usefulness in extending the shelf life of the two types of palm wine by offering cheaper preservative means of extending the shelf-life of locally tapped palm wine. Further developments as a possible avenue to strengthen the shelf life extension methods employed in palm wine preservation without affecting the taste and acceptability could be achieved, thus contributing to the search for low cost preservative methods for palm wine storage.

## Conflict of Interest

Authors have declared that no competing interests exist.

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