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Research Article
Effect of Bitter Leaf Extract (*Vernonia amygdalina*) on Culturable Microorganisms Isolated from Palm Wine in Makurdi Metropolis

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Abstract

The bio-preservative effects of leaf of bitter leaf extract (*Vernonia amygdalina*), respectively on 2 palm wine types namely, palm wine with bitter leaf extract and palm wine without bitter leaf extract from Makurdi, Nigeria was evaluated. Samples were analysed by adding 1 mL of bitter leaf extract into 1000 mL of palm wine and a control of 1000 mL without bitter leaf extract was monitored at interval of 12 h. The microbiological and biochemical changes of the palm wine brands were determined, palm wine without bitter leaf extract were found to support more heterotrophic and coliform populations than the palm wine with bitter leaf extract, while the later contained more yeast species. Identification of isolated species revealed the presence of *Bacillus* sp., *Micrococcus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Bacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Zygomonas* sp., *Neurospora* sp., *Aspergilus niger*, *Aspergilus fumigate* and *Penicillium notatum*. The time, frequency and percentage of palm wine sample without bitter leaf extracts on nutrient, MacConkey and sabouraud destrose agar and their CFU mL^{-1} shows the highest at total time of 156 h has the frequency of 73, the percentage was 100% with the total microbial count of 73.0×10^5 CFU mL^{-1}, while palm wine with bitter leaf extract show the lowest at total time of 156 h has the frequency of 43, the percentage was 100% with the total microbial count of 43.0×10^5 CFU mL^{-1}. The one with bitter leaf extract have lower microbial count because bitter leaf extract (*V. amygdalina*), lowered the bacterial and fungal growth because its serve as an antimicrobial agent that inhibit microbial growth.

Key words: Palm wine, microbial count, bitter leaf extract

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Palm wine is the fermented sap of various species of palm trees such as *Elias guineensis*, *Raphia regalis*, *Raphia sudanica*, *Raphia* vanifera and *Raphia hookeri* (Obire, 2005). Palm wine can be obtained from the young inflorescence either male or female palm tree. 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 very short shelf life. The wine is consumed in a variety of flavours varying from sweet unfermented to sour, fermented and vinegary palm wine.

Palm wine has been used locally in Nigeria for ethanol production by rural farmers. In Nigeria, it is abundant in the Niger delta and Rivers state in particular. It is common in Benue state, especially among the Idoma speaking communities as it is found in large quantities in Ogbadibo and Okpokwu local governments. The palm wine consumed within Makurdi metropolis is gotten from the above local governments. The sap is extracted and collected by a tapper. Typically, the sap is collected from the cut flower of the palm tree. A container is fastened to the flower stump to collect the sap. The white liquid that initially collects, tends to be very sweet and non-alcoholic before it is fermented. The saps, which are rich in sugars, are fermented naturally by yeasts of the genera Saccharomyces. Lactic acid bacteria have also been implicated to contribute to the characteristic flavour of fresh palm wine (Okafor, 2007).

The importance of palm wine especially in African societies extends across the cultural and medical device. A litre of palm wine contains approximately 300 call, 0.5-2.0 g of proteins and a considerable amount of vitamins mainly vitamin A, C and K, which helps in protecting and improving the consumer's eye sight (Nester *et al*., 2004). Palm wine is also the wine of choice at traditional wedding ceremonies among the Igbo and Yoruba, a bride confirms a bridegroom as her husband by pretending to seek him out among the crowd and kneeling down to symbolically present a calabash of palm wine to him (Iheonu, 2000). Palm wine is also the drink of choice at traditional religious ceremonies or festivals, wines are offered to the ancestors as a sign of worship. In addition drinking, palm wine is powerful enough to fight pneumonia and palm sugar has the properties to cure fever and stomach pain (Okafor, 1978). Palm wine and its distillate are important solvent in herbal-medicinal administration; pregnant women consume it fresh for the sweetness and nutrition, while nursing mothers drink it warm to enhance breast milk production. Palm wine serves as a source of innocula for other fermentations. In Asia, it is the source of inoculums for cottage industry fermentations, such as Nan (a native leavened bread) and Sonnon (steamed rice flours plus palm wine) (Batra and Milner, 1974). Also, Palm wine yeast is found capable of degrading hydrocarbons in kerosene and diesel (oil spills), confirmatory evidence was derived from gas chromatographic analysis. Yeast used hydrocarbons as a carbon source and energy source for growth, which suggest the isolates potential application in oil spill cleanup as well as in single cell protein production using hydrocarbon feed stocks (Amanchukwu *et al*., 1989).

Fermentation of palm wine is the metabolic conversion of carbohydrates contained in the wine, such as sugar into alcohol or an acid using yeast, bacteria or a combination thereof (mixed culture) (Adams and Moss, 1995). It is also the slow decomposition of organic substance induced by micro-organisms or by complex nitrogenous substances (enzymes) of plant and animal origin (Board, 1983). In this process, also starch contained in the wine is broken down into fermentable sugars by fungal enzymes such as α amylase and glucoamylase to facilitate fermentation by yeast, mainly *Saccharomyces* species. Fermentation of palm wine could occur under anaerobic or aerobic conditions and yields lactate, acetic acid, ethanol, carbon dioxide or some other simple product (Esechie, 1979).

The numerous important products obtained by fermentation such as antibiotics, vitamins, feed supplements and blood plasma expanders, has made fermentation as an industrial method for making specialty and various industrial chemicals gain wide attention (Horold *et al*., 1981).

Vernonia amygdalina commonly called bitter leaf is the most widely cultivated species of the genus Vernonia, which has about 1,000 species of shrubs (Munaya, 2013). It belongs to the family Astaraceae. It is popular in most West African countries including Nigeria, Cameroon, Gabon and Congo Democratic Republic.

Palm wine contain large numbers of microorganisms including bacteria, yeast and moulds (Okafor, 1978). Some of these microorganisms are found in palm wine as a result of improper handling by the tappers and marketers and this can be harmful for human consumption. Over fermented palm wine reputed to cause disease or at least to be closely linked to diseases, such as diarrhoea and headaches. The microbial cause of this spontaneous fermentation has always been wondered for scientist and the general public. A comparative study of the bacteria isolated and characterized from and fermented palm wine will give a better understanding of the variation in microbial species of the palm wine samples, which

may be accountable for the souring of the palm wine due to fermentation. Knowing the bacteria responsible for fermentation of the palm wine, will also help in ascertaining the preservation technique to be uses in improving the shelf life and contamination combat the spontaneous fermentation. The result may be used to improve in ways to preserve palm wine consumed in Makurdi.

This study is therefore, aimed at ascertaining microbial and biochemical changes associated with non-preserved and bio-preservative effect of *V. amygdalina* (bitter leaf) on palm wine in Makurdi metropolis. The objectives of the study were:

- To isolate, characterize and identify the microorganisms present in palm wine
- To determine the effect of bitter leaf on the isolates from palm wine

MATERIALS AND METHODS

The study was carried out at Advanced Biological Science Laboratory of the Federal University of Agriculture, Makurdi, Benue State, Nigeria. Makurdi is located in Central Nigeria along the Benue river with an estimated population of 500, 797. The town has an annual rainfall of 1099 mm with dry and rainy seasons and the temperature ranges from 27-35°C and it coordinate is 7°44°N latitude and 8°536°E longitude in the tropical guinea Savannah flood plain of river Benue.

Materials: The glass wares used include petri-dishes, test-tubes, pipette, conical flasks and beakers. These were washed with detergent and rinsed well with clean water. They were then sterilized in the oven at 160°C for 1 h The media used include Nutrient Agar (NA), Sabouraud's Dextrose Agar (SDA) and distilled water. These were prepared according to manufacturer's specification and sterilized in the autoclave at 121°C for 15 min before use. Other materials used include hydrogen peroxide, syringes, centrifuge and ethanol.

Sample collection: Fresh palm wine sample from oil palm tree (*E. guineensis*) was collected from traditional palm wine collectors from North bank Makurdi Benue state, Nigeria. The freshly tapped sample was collected using a total of 10 pre-sterilized 1000 mL capacity bottles with screw caps. The perforated screw caps were plugged with sterile non-absorbent cotton wool and 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.

Plant materials: Leaves of *Vernonia amygdalina* (bitter leave) collected from Makurdi Benue state, Nigeria. The plant materials were washed with sterilized water, to avoid contamination.

Microbiological analysis serial dilution: About 1 mL aliquots of each palm wine sample was taken aseptically at 0, 24, 48, 72, 96 and 120 h of fermentation and 24 h of fermentation and were serially diluted 5-folds in 0.1% (w/v) distilled water. About 1 mL dilutions were plated out using pour plate, nutrient agar for total bacterial count, MacConkey agar for the total coliform count and potatoes dextrose agar containing 0.05 mg mL^{-1} chloramphenicol for yeast count as 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^{-1}. They were stored on agar slants at 40°C for characterization.

Biochemical analysis of the 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. Bacteria isolates were thereafter subjected to catalase, coagulase and oxidase tests as described by Cheesbrough (2002).

Gram staining: The bacterial cells were first heat fixed and then stained with a basic dye, crystal violet, which is taken up in similar amounts by all bacteria. The slides were then treated with Lugol's iodine (mordant) to fix the stain, washed briefly with 95% alcohol (de-stained) and finally counter stained with a paler dye of different color (safranin). Gram-positive organisms retain the initial violet stain, while Gram-negative organisms are decolorized by the organic solvent and hence, show the pink counter stain. The difference between Gram-positive and Gram-negative bacteria lies in the ability of the cell wall of the organism to retain the crystal violet.

Statistical analysis: Data obtained were analysed using descriptive statistics, SPSS version 20.

RESULTS

One thousand milliliter of palm wine was obtained and was cultured on nutrient agar, MacConkey agar and potatoes dextrose agar for both bacteria and fungi. The cultured plate were further analyzed by observing the morphological characteristics, Gram staining and other biochemical test

Table 1: Morphological and biochemical characteristics of bacteria isolated from palm wine samples without bitter leaf extract

Morphological characteristic	Gram rxn	CAT	COA	OXI	Isolated organism
Gc	+ve cocci in chain -ve rod	-	-	-	*Streptococus Pneumoniae*
Wc	-ve rod	+ve	+ve	-ve	Pseudomonas
Cc	+ve rod	-ve	+ve	-ve	*Bacillus* sp.
Syc	+ve cocci in cluster	+ve	-ve	+ve	*Stapylococus aureus*
Pc	+ve cocci	+ve	-ve	-ve	*Micrococcus* sp.
Smc	+ve rod	+ve	-ve	-ve	*Lactobacillus* sp.

+ve: Positive, -ve: Negative, Gc: Greenish colonies, Wc: Whitish colonies, Cc: Creamy colonie, Syc: Small yellowish colonies, Pc: Pinkish colonies, Smc: Small milky colonies, rxn: Reaction, CAT: Catalase, OXI: Oxidase and COA: Coagulase

Table 2: Morphological and biochemical analysis of organism isolate from palm wine sample with bitter leaf

Morphological characteristics	Gram rxn	CAT	COA	OXI	Isolated organism
CC	+ ve rod	-ve	+ve	-ve	*Bacillus* Spp.,
PC	+ve cocci	+ve	-ve	-ve	*Micrococcus* Spp.,
Smc	+ve rod	+ve	-ve	-ve	*Lactobacillus* Spp.,

+ve: Positive, -ve: Negative, Cc: Creamy colonies, Pc: Pinkish Colonies, SmC: Small yellowish Colonies, CAT: Catalase, COA: Coagulase and OXI: Oxidase

Table 3: Time frequency and percentage of palm wine sample with bitter leaf extract on NA, Mac A and PDA and their CFU mL^{-1}

Reagents	Time (h)	Frequency	Percentage	CFU mL^{-1}
NA	0	2	4.6	2.0×10^5
Mac A	0	2	4.6	2.0×10^5
PDA	0	1	2.3	1.0×10^5
NA	12	7	16.2	7.0×10^5
Mac A	12	8	18.6	8.0×10^5
PDA	12	1	2.3	1.0×10^5
NA	24	5	11.6	5.0×10^5
Mac A	24	4	9.3	4.0×10^5
PDA	24	1	2.3	1.0×10^5
NA	48	3	6.9	3.0×10
Mac A	48	3	6.9	3.0×10^5
PDA	48	1	2.3	1.0×10^5
NA	72	2	4.6	2.0×10^5
Mac A	72	2	4.6	2.0×10^5
PDA	72	1	2.3	1.0×10^5
	156	43	100.0	43.0×10^5

NA: Nutrient agar, Mac A: MacConkey agar and PDA: Potatoes dextrose agar

Table 4: Time frequency and percentage of palm wine sample without bitter leaf extract on NA, Mac A, PDA and their CFU mL^{-1}

Reagents	Time (h)	Frequency	Percentage	CFU mL^{-1}
NA	0	2	2.7	2.0×10^5
Mac A	0	2	2.7	2.0×10^5
PDA	0	2	2.7	2.0×10^5
NA	12	5	6.8	5.0×10^5
Mac A	12	6	8.2	6.0×10^5
PDA	12	1	1.4	1.0×10^5
NA	24	7	9.6	7.0×10^5
Mac A	24	7	9.6	7.0×10^5
PDA	24	2	2.7	2.0×10^5
NA	48	8	10.9	8.0×10
Mac A	48	8	10.9	8.0×10^5
PDA	48	2	2.7	2.0×10^5
NA	72	9	12.3	9.0×10^5
Mac A	72	9	12.3	9.0×10^5
PDA	72	3	4.1	3.0×10^5
	156	73	100.0	73.0×10^5

NA: Nutrient agar, Mac A: MacConkey agar and PDA: Potatoes dextrose agar

and the following results were obtained as shown in Table 1-6. Table 1 shows the morphological and biochemical characteristics of organism isolated from palm wine without bitter leaf extract. Table 2 highlights the morphological and biochemical characteristics of organism isolated from palm wine with bitter leaf. Time-dependent variation, frequency distribution and percentage occurrence of the isolates from palm wine sample treated with bitter leaf extract on NA, Mac A and PDA and their CFU mL^{-1} are as shown in Table 3. Time, frequency and percentage of palm wine sample without bitter leaf on NA, Mac A and PDA and their CFU mL^{-1} is as shown in Table 4. Table 5 shows the cultural and microscopic characteristics of fungi isolated from palm wine sample with bitter leaf extract in PDA. Table 6 shows the cultural and microscopic characteristics of fungi isolated from palm wine sample without bitter leaf extract on PDA.

DISCUSSION

The microbiological assays revealed that more total heterotrophic bacteria and coliform counts were obtained from palm wine without bitter leaf extract compare to the one with bitter leaf extract, while the later had more yeast counts than the former. The isolated microorganisms were *Bacillus* sp., *Micrococcus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Bacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Zygomonas* sp., *Neurospora* sp., *Aspergilus niger*, *Aspergilus fumigate* and *Penicillium notatum*. Previous studies of Onwuakor and Ukaegbu-Obi (2014) isolated *Bacillus* sp., *Micrococcus* sp., *Lactobacillus* sp., *Brevibacterium* sp. and *Saccharomyces* sp., which corroborate the findings. Environmental factors, method of palm wine extraction, nature of containers, hygienic practices and exposure conditions may account for presence of microorganisms implicated in this study other than the ones reported in previous studies.

Table 5: Cultural and macroscopic characteristic of fungi isolated from palm wine samples (without bitter leaf extract) on potatoes dextrose

Microscopic morphology	Isolated organism
It is whitish in colour and wooly in nature	*Neurospora crassa*
It is creamish in colour and in shape and sometimes white in colour	*Sacharomyces cerevisiae*
Black mycelia growth and fully extended from the growth medium	*Aspergillus niger*
Brown mycelia growth and put conidiosphores	*Aspergillus fumigates*
The texture have a layer of soft short hairs and the appearance is grayish to green	*Penicillum notanum*

Table 6: Cultural and microscopic characteristic of fungi isolated from palm wine samples with bitter leaf on sabouraud dextrose

Microscopic morphology	Isolated organism
Black mycelia growth and fully extended from the growth medium	*Aspergillus niger*
It is whitish in colour and wooly in nature	*Neurospora crassa*

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 samples, while coliform counts gradually increased up to 72 h. Yeast population showed a steady increase from 24 h of fermentation to the 72 h. Chandrasekhar *et al*. (2012) opined that wine serve as a good substrate for microbial growth though the natural microbial flora is bound to rapidly decrease, as the wine is converted to alcohol and other products. A sharp progressive decrease was observed from 72 h by all the palm wine samples. This sharp decrease varies with previous studies of Onwuakor and Ukaegbu-Obi (2014), which reported a gradual loss of bacterial and fungal viability with increased fermentation from 48-120 h, which study attributed to loss of sugar, production of organic acids and reduction in pH. Generally, samples of palm wine without bitter leaf extract, were observed to have higher heterotrophic bacteria, coliform and yeast counts compared to the samples with bitter leaf extract (*V. amygdalina*) as a preservative. The use of extracts of *V. amygdalina* could be responsible since it is reported to have bioactive components with antimicrobial properties (Akujobi *et al*., 2004). Microbial populations of palm wine can be inactivated with the use of extracts of *V. amygdalina* as a preservative, thereby extending its shelf life and justifying its traditional use.

In this study, palm wine samples that were not treated with bitter leaf at 0 h on nutrient agar had a bacterial frequency of 2, with a percentage of 4.6% and the total bacterial counts was 2.0×10^5 CFU mL^{-1} in palm without preservative is still the same, because the palm wine is still fresh at 0 h. At 12 h in palm wine with bitter leaf extract had, the frequency of 5 with a bacterial count of 5.0×10^5 CFU mL^{-1}, while at 12 h in palm wine, without bitter leaf extract had the frequency of 7 with a bacterial count of 8.0×10^5 CFU mL^{-1} at 24 h. Palm wine samples treated with bitter leaf had the frequency of 5 with a bacterial count of 5.0×10^5 CFU mL^{-1}, while at 24 h in palm wine without bitter leaf extract had the frequency of 7 with a bacterial count of 7.0×10^5 CFU mL^{-1}. At 48 h in palm wine, with bitter leaf extract had the frequency of 3 with a bacterial count of 3.0×10^5 CFU mL^{-1}, while at 48 h in palm wine without bitter leaf extract had the frequency of 8 with a bacterial count of 8.0×10^5 CFU mL^{-1} and at 72 h in palm wine with bitter leaf extract had the frequency of 2 with a bacterial count of 2.0×10^5 CFU mL^{-1}, while at 72 h in palm wine without bitter leaf extract had the frequency of 9 with a bacterial count of 9.0×10^5 CFU mL^{-1} and a fungi count of 3.0×10^5 CFU mL^{-1}. The total bacterial counts were relatively low in palm wine samples treated with *V. amyglalina* (bitter leaf) compared to those without preservative. The isolation and identification of these microbial species from fermenting palm wine show the presence of different microbial flora of exposed fermentary palm wine. Probable microbial isolates were identified as *Bacillus* sp., *Micrococus* spp., *Saccharomyces*, *Lactobacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Zygomonas* sp., *Bacillus* sp., *Neurospora* sp., *Aspergillus niger*, *Aspergillus fumigate* and *Penicillium notatum*. Studies of Okolie *et al*. (2013) in their previous report on evaluation of bacterial diversity in palm wine by 16S rDNA analysis of community DNA revealed that 32 community clones were identified as *Lactobacillus* sp., *Lactobacillus casei* strain Zhang, *Lactobacillus plantarum*, *Leuconostoc mesenteriodes* sp., *dextranicum*, *Leuconcostoc lactis*, *Pediococcus parvulus* strain Bpe-299, *Acetobacter pomorum*, *Acetobacter pasteurianus*, *Gluconobacter oxydans*, *Acinetobacter calcoaceticus*, *Enterobacterium bacterium*, *Acidovorax* sp., *Comamonas* sp., *Bacillus subtilis*, *Staphylococcus piscifermentans* and uncultured bacteria clone D1-78. The results showed that bacterial diversity in the palm wine sample is dominated by Lactobacillus and Leuconostoc species as reported by previous workers and uncultured bacteria clone D1-78 (1 clone) was detected for the first time in palm wine. Bacteria from the genera Lactobacillus, Leuconostoc, Pediococcus and Acetobacter have been identified earlier in previous studies of palm wine fermentation using traditional cultivating

technique (Okafor, 1975, Bassir, 1968; Uzochukwu *et al*., 1994a, b). Ezeigbo *et al*. (2014) had isolated and identified microorganisms from fermented palm wine based on their cultural, morphological and biochemical characteristics. The microorganisms included *Lactococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Streptococcus* spp., *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli*, *Sacchromyces* spp. and *Candida* spp. *Sacchromyces* spp., occurred in all the samples (100.0% occurrence). The mean average microbial counts of the samples in their report were 3.2×10^4, 2.0×10^4 and 0.4×10^4 CFU mL^{-1} for fungi, heterotrophic and coliform bacteria counts, respectively. Other studies implicated *Sacchromyces cerevisae* and *Candida albicans* (Nester *et al*., 2004; Chandrasekhar *et al*., 2012) and bacteria encountered in palm wine and reported were Lactobacillus, Leuconostoc, Bacillus, Streptococcus, Zymomonas, *E. coli*, Brevibacterium, Micrococcus, Peliococcus, Corynebacterium, Klebsiella, Peptostreptococcus, Gluconobacter and Chomobacterium (Bassir, 1962; Okafor, 1978; Nwachukwu *et al*., 2006).

The presence of *Bacillus* spp., *Staphylococcus* spp. and *Candida* spp. in this study is an indication of the unhygienic handling of the beverage and this could be harmful to the consumers. The isolation of *Micrococcus* sp., from fermenting palm wine poses health implication, which might have been due to exposure of freshly tapped wine that show various forms of pathogenic bacteria associated with exposed palm wine. The frequent gastro intestinal problem associated with drinking palm wine well over 24 h could be attributed to the presence of pathogenic bacteria in palm wine. Present study, urged appropriate sanitation and quality assurance and control agencies to ensure that proper personal and environmental hygiene is enforced to prevent public health problems.

CONCLUSION AND FUTURE RECOMMENDATION

This study, therefore showed that the use of *V. amgdalina* leaves (bitter leaf) 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 development 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 preservation methods for palm wine storage.

- Palm wine contains pathogenic micro organisms as a result of contamination, therefore it should be properly handled with care during handling and retailing
- Tapers should improve on their personal hygiene
- Palm wine contain organic acid like lactic acid and acetic acid at some level of fermentation and should not be taken by ulcer patients because if taken, increases the pain of the wound like any other acidic drugs
- Palm wine should be package like any other alcoholic beverages to prevent contamination and increase the shelf life
- Identification of the bioactive components of *V. amygdalina* with antimicrobial properties should be identified and processed at industrial scale for use as preservatives

REFERENCES

Adams, O. and K. Moss, 1995. Handbook of Indigenous Fermented Foods. Marcel Decker Inc., New York, USA., pp: 59-60.

Akujobi, C., B.N. Anyanwu, C. Onyeze and V.I. Ibekwe, 2004. Antibacterial activities and preliminary phytochemical screening of four medicinal plants. J. Applied Sci., 7: 4328-4338.

Amanchukwu, S.C., A. Obafemi and G.C. Okpokwasili, 1989. Single-cell-protein production by *Schizosaccharomyces pombe* isolated from palmwine using hydrocarbon feedstocks. Foliar Microbiolol., 34: 112-119.

Bassir, O., 1962. Observations on the fermentation of palm wine. West Afr. J. Biol. Applied Chem., 6: 20-25.

Bassir, O., 1968. Some Nigerian wines. W. Afr. J. Biol. Chem., 10: 42-45.

Batra, L.R. and P.D. Milner, 1974. Some Asian fermented foods and beverages and associated fungi. Mycologia, 66: 942-950.

Board, M., 1983. Fermented Beverage Production. Blackie Academic and Professional Press, pp: 67-96.

Chandrasekhar, K., S. Sreevani, P. Seshapani and J. Pramodhakumari, 2012. A review on palm wine. Int. J. Res. Biol. Sci., 2: 33-38.

Cheesbrough, M., 2002. District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, UK., pp: 135-162.

Esechie, H.A., 1979. Fermentation studies on Nigeria palm-wine. Niger. Agric. J., 16: 96-102.

Ezeigbo, O.R., N.G. Agomoh, S. Uhiara, M.U. Ajuga and U.P. Okeke, 2014. Microbiological analysis of fermented palm wine sold in Aba South LGA, Abia state, Southeastern Nigeria. Standard Res. J. Microbiol. Sci., 1: 39-42.

Horold, H., J. Smith and L. Octave, 1981. Classification of foods. J. Food Sci. Technol., 4: 13-21.

Iheonu, T.E., 2000. Effect of local Preservatives of plant Origin on Microbiology and shelf life stability of palm wine. B.Sc. Thesis, Abia State University, Nigeria

Munaya, C., 2013. Bitter leaf-based extracts cures hepatitis co-inferation and others. The Guardian Newspaper, July, 25, 2013.

Nester, E.W., D.G. Anderson, C.E. Roberts, N.N. Pearsall and M.T. Nester, 2004. Microorganisms in Food and Beverage Production-Alcoholic Fermentation by Yeast. In: Microbiology: A Human Perspective, Nester, E.W. (Ed.). 4th Edn., McGraw-Hill, New York, USA., ISBN-13: 9780071214933 pp: 151-153.

Nwachukwu, I.N., V.I. Ibekwe and B.N. Anyanwu, 2006. Investigation of some physicochemical and microbial succession parameters of palm wine. J. Food Technol., 4: 308-312.

Obire, O., 2005. Activity of *Zymomonas* species in palm-sap obtained from three areas in Edo state, Nigeria. J. Applied Sci. Environ. Manage., 9: 25-30.

Okafor, N., 1975. Preliminary microbiological studies on the preservation of palm wine. J. Applied Bacteriol., 38: 1-7.

Okafor, N., 1978. The biochemistry and microbiology of palm wine. Adv. Applied Microbiol., 24: 237-256.

Okafor, N., 2007. Palm Wine. In: Modern Industrial Microbiology and Biotechnology, Okafor, N. (Ed.). Science Publishers, USA., ISBN: 9781578084340, pp: 270-271.

Okolie, P.I., C.N. Opara, E.C. Emerenini and S.V.A. Uzochukwu, 2013. Evaluation of bacterial diversity in palm wine by 16S rDNA analysis of community DNA. Niger. Food J., 31: 83-90.

Onwuakor, C.E. and K.M. Ukaegbu-Obi, 2014. 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. Am. J. Microbiol. Res., 2: 113-117.

Uzochukwu, S.V.A., E. Balogh, O.G. Tucknot, M.J. Lewis and P.O. Ngoddy, 1994a. Volatile constituents of palm wine and palm sap. J. Sci. Food Agric., 64: 405-411.

Uzochukwu, S.V.A., P.O. Ngoddy and E. Balogh, 1994b. The role of microbial gums in the color and consistency of palm wine. J. Food Qual., 17: 393-407.