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Chemical Composition of Some Natural Palm Wine Preservatives

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ABSTRACT

Palm wine is the commonest name of the beverage obtained from fermented palm sap, the exudate from tapped unopened spathe of oil palm tree (Elaeis guineensis). This refreshing wine of West and Central Africa is very sweet but within 24 hours the concentration of sucrose falls to less than 50% the initial amounts due to a rapid sugar fermentation by microorganisms. In Cameroon, Central Africa, traditional attempts to preserve palm wine imply the introduction of natural preservatives mostly barks and leaves from edible or medicinal plants. The leaves of Cymbopogon citratus, Pimenta racemosa, Vernonia amygdalina, Ocimum basilicum and the barks and the leaves of Garcinia lucida and of Adansonia digitata commonly used in palm wine preservation were analyzed for their chemical composition. The results showed that lipid, protein, ash, crude fiber, total sugars and vitamin C contents were respectively from 15.70 ± 1.13 to 23.57 ± 1.80%, from 8.38 ± 0.38 to 43.81 ± 3.69%, from 1.14 ± 0.17 to 10.06 ± 0.39%, from 11.74 ± 1.11 to 48.42 ± 0.55%, and from 1.49 ± 0.02 to 22.56 ± 0.59% and from 55.76 ± 3.15 to 175.71 ± 2.09 µg /100 g dw. The phyto-chemicals levels were respectively from 1.37 ± 0.22 to 3.61 ± 0.6 % for total alkaloids, 2.66 ± 0.12 to 4.80 ± 0.24 % for total phenolics and 0.57 ± 0.09 to 1.88 ± 0.09 mg /100 g dw for total saponins. Concerning anti-nutrients, the oxalates levels were higher than the threshold value (250 mg/100 g) reported as safety limit. Despise the proven antimicrobial benefits of alkaloids, saponins and phenolics found in those leaves and barks, caution shall be paid during their use due to their high oxalates levels.

KEYWORDS: Palm wine, Natural preservatives, *Cymbopogon citratus*, *Pimenta racemosa*, *Vernonia amygdalina*, *Ocimum basilicum*, *Garcinia lucida*, *Adansonia digitata*, oxalates.

INTRODUCTION

The unfermented exudate from tapped unopened spathe of oil palm tree (*Elaeis guineensis*) is referred to as palm sap. It is widely consumed as a refreshing beverage in West and Central Africa [1]. The fermented palm sap, called palm wine is the commonest form of the beverage. Before, the consumption of the beverage (palm sap/wine) was a common feature in virtually all ceremonies (traditional festivals, weddings and funerals) where it was served as an indication of hospitality [2]. Nowadays, the drink is becoming more and more present in the diet as it is supplied daily in restaurant or sold in the street.

Fresh palm wine is very sweet and refreshing because of the presence of sucrose, but within 24 hours the concentration of sucrose falls to less than 50% the initial amounts due to a rapid sugar fermentation by microorganisms. Fermentation virtually ends when the pH falls to 4.0; the whole process lasts about 48 hours resulting in an undesirable sour drink. Microorganisms reported in palm wine include Bacillus, Streptococcus, Saccharomyces, Schizosaccharomyces, Pischia, Leuconostoc, Micrococcus, Serratia, Aerobacter, Pseudomonas, Cornybacterium, Asppergillus, and Candida [1].

In Cameroon, traditional attempts to preserve palm wine imply the introduction of natural preservatives mostly barks and leaves from edible or medicinal plants like *Vernonia amygdalina*, *Cymbopogon citratus*, etc. So, palm wine is supplied and drank with those preservatives inside. Due to their chemical composition, these preservatives offer chemical compounds (essential oils, alkaloids, phenolic compounds, etc.) preventing the microbial activity [3,4] and therefore increasing the shelf life of palm wine.

Besides bioactive compounds, those preservatives also provide nutrients and antinutrients influencing therefore the nutritional quality of palm wine. However, to our knowledge, little information is available on the chemical composition of those barks and leaves. This study aims to assess the nutritional composition of some barks and leaves used in palm wine preservation as well as their bioactive compounds.

MATERIALS AND METHODS

Procurement of leaves and barks

The samples used for the experiment were the leaves of *Cymbopogon citratus* (Citronella), *Pimenta racemosa* (bay leaf), *Vernonia amygdalina* (bitter leaf), *Ocimum basilicum* (Massep); the barks and the leaves of *Garcinia lucida* (Essok) and of *Adansonia digitata* (baobab). The samples were purchased at Mfoundi market and in a farm at Nkolbisson (Yaounde, Cameroon). Leafy stem specimen collected from the samples were identified at the Cameroonian National Herbarium.

Preparation of samples

Fresh leaves and barks were selected, washed and dried at 50 °C. Then, the samples were grinded and sieved to obtain a fine powder whose particle size was 500 µm.

Evaluation of nutritional composition

Evaluation of proximate composition

Moisture content was determined after oven drying to a constant weight at 105 °C. Ash, proteins, lipids and crude fibers were analyzed according to AOAC methods [5,6].

Determination of ascorbic acid level

An amount of 0.5 g was stirred twice during 15 min in presence of 10 mL 90% acetic acid, then filtrated into 20 mL standard flask and the volume was adjusted to the mark with 90% acetic acid. About 0.5 mL of filtrate was mixed with 4.5 mL of 50 µmol/L 2.6 dichloroindophenol sodium salt hydrate and the absorbance was measured within 30 min at 515 nm. Ascorbic acid content was calculated on the basis of the calibration curve of L-ascorbic acid ranging from 0.2 to 0.4 µg /mL [7].

Determination of antinutrients

Determination of oxalates content

A specimen (2 g) was digested with 10 mL of 6 M hydrogen chloride and 190 mL of distilled water. The filtrate was put in a conical flask of 250 mL with distilled water. The pH of the solution was increased using liquid ammonia until the color moved from salmon pink to fair yellow then, the filtrate was treated with 10 mL of 5 % chloride of calcium for oxalate precipitation. Afterwards, the mixture which was allowed to settle for 24 h was centrifuged and the precipitate dissolved in 10 mL of 20 % sulphuric acid. The total filtrate resulting from the dissolution in 20 % sulphuric acid was completed to 300 mL with distilled water. An aliquot of 125 mL was heated until boiling and titrated with potassium permanganate 0.098 N until a constant pink during 30 s [8].

Determination of hydrogen cyanide content

Hydrogen cyanide content was determined as described by [9]. Sample (1 g) was soaked in a mixture of 200 mL of water and 10 mL of orthophosphoric acid. The mixture was left 12 h to release all bounded hydrocyanic acid. Antibumping agents were added, the solution distilled and the distillate measured. A volume of 10 mL of distillate was taken into a conical flask and diluted with 20 mL of water, 4 mL of 6 M ammonia and 1 mL 5 % potassium iodide. The mixture was titrated with 0.02 M silver nitrate until a faint but permanent turbidity was

obtained. The hydrogen cyanide contents were obtained using the relationship (1 mL 0.02 M AgNO3= 1.08 mg HCN).

Determination of phytates content

An amount of 150 mg of sample was extracted with 5 mL of 2.4 % hydrochloric acid during 2 h. A volume of 50 µL of extract was added to 750 µL of Wade solution (0.15 g of sulfosalicylic acid and 15 mf of ferric chloride in 50 mL of water) and the absorbance was read at 500 nm. The calibration curve of phytic acid ranged from 70 to 300 µg /mL[10].

Estimation of phytochemicals

Determination of alkaloids content

A total of 20 mL of 20 % acetic acid was added to 0.5 g of sample taken in a separate 250 mL beaker and covered to stand for 4 h. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample, concentrated ammonium hydroxyde was added drop-wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed [11].

Estimation of total saponins content

Estimation of total saponins content was determined by the method described by [12]. A mass of 0.5 g of sample was extracted twice with 25 mL of ethanol 80 % for 30 min. About 50 µL of extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5 % ethanol) was added. Then 2 mL of 72 % sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 °C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as mg saponin equivalent (mg SE / of 100g) derived from a standard curve.

Determination of total phenolics content

About 0.5 g of sample was introduced into a round bottom flask and extracted twice with 25 mL of ethanol 80 % on a magnetic stirrer for 30 min. After this, the mixtures were filtered with a Wathman filter paper Nº 1 and the filtrates were poured in 50 mL standard flasks and the volume were adjusted to the mark using ethanol 80 %. To 125 µL of extract, were added 0.5mL of distilled water and 125 µL of the Folin Ciocalteau solution (1:16). After 6 min, 1250 µL of 7% sodium carbonate were added and the volume was adjusted to 3 mL with distilled water and the absorbance measured after 90 min against a blank tube containing extracting solvent at 760 nm. The calibration curve of gallic acid ranged from 1 to 5 µg /mL [13].

RESULTS AND DISCUSSION

Evaluation of nutritional composition

The nutritional composition of palm wine preservatives was presented in table 1. As displayed in table 1, in the eight leaves and barks used for palm wine preservation, the lipids content ranged from 15.70 ± 1.13 to 23.57 ± 1.80 g /100 g dw; the minimum level was found in *Vernonia amygdalina* leaves and the maximum in *Garcinia lucida* leaves. This lipids content found in *Vernonia amydalina* leaves (15.70 ± 1.13 g /100 g dw) was higher than 11.3 and 6.04% reported respectively by [4,14] to be the lipids content of *Vernonia amydalina* leaves.

The ash content of leaves and barks under study varied from 1.14 ± 0.17 g /100 g dw in *Garcinia lucida* bark to 10.06 ± 0.39 g /100 g dw in *Vernonia amygdalina* leaves. The concentrations of ash found in *Vernonia amygdalina* leaves (10.60 ± 0.39 g /100 g dw) and in *Cymbopogon citratus* leaves (7.87 ± 0.10 g /100 g dw) were consistent with the levels found respectively in *Vernonia amygdalina* (11.8%) by [4] and in *Cymbopogon citratus* (7.15 ± 0.21%) by [15].

Table 1. Nutritional composition of some palm wine preservatives

Plants	Part	**Lipids (g /100 g dw)**	**Ash (g /100 g dw)**	**Total sugar (g /100 g dw)**	**Crude fibers (g /100 g dw)**	**Crude proteins (g /100 g dw)**	**Ascorbic acid (µg /100 g dw)**
Vernonia amygdalina	Leaf	15.70 ± 1.13^{a}	10.60 ± 0.39^{a}	3.08 ± 0.01^{a}	16.97 ± 1.11^{a}	43.81 ± 3.69^{a}	118.36 ± 8.37^{a}
Cymbopogon citratus	Leaf	19.94 ± 0.10^{b}	7.87 ± 0.10^{b}	22.56 ± 0.59^{b}	38.12 ± 0.36^{b}	14.06 ± 0.88^{b}	104.05 ± 2.13^{b}
Pimenta racemosa	Leaf	18.82 ± 0.91^{c}	3.30 ± 0.07^{c}	1.69 ± 0.18^{c}	38.91 ± 0.55^{b}	14.00 ± 1.56^{b}	153.81 ± 4.27^{c}
Ocimum basilicum	Leaf	19.05 ± 0.12^{c}	10.05 ± 1.10^{a}	2.97 ± 0.18^{a}	11.74 ± 1.11^{c}	29.94 ± 2.31^{c}	87.71± 12.83^{d}
Adansonia digitata	Bark	17.44 ± 0.71^{d}	7.90 ± 1.90^{bd}	1.89 ± 0.08^{c}	42.84 ± 1.75^{d}	7.88 ± 0.50^{d}	92.61 ± 4.22^{e}
Adansonia digitate	Leaf	22.17 ± 2.72^{bd}	5.52 ± 1.05^{d}	1.49 ± 0.02^{c}	48.42 ± 0.55e	21.88 ± 0.95^{e}	55.76 ± 3.15^{f}
Garcinia lucida	Bark	16.28 ± 0.20^{a}	1.14 ± 0.17^{e}	2.12 ± 0.07^{d}	20.37 ± 0.35^{f}	10.00 ± 0.13^{f}	83.41 ± 8.43^{e}
Garcinia lucida	Leaf	23.57 ± 1.80^{d}	4.22 ± 0.69^{f}	3.46 ± 0.11^{e}	20.37 ± 0.35^{f}	8.38 ± 0.38^{d}	175.71 ± 2.09^{g}

Means values within a column having differing superscripts are significantly different at $P < 0.05$

Concerning the crude fibers content of leaves and barks analyzed, the values ranged from 11.74 ± 1.11 to 48.42 ± 0.55 g /100 g dw. The crude fibres level observed in *Cymbopogon citratus* (38.12 ± 0.36 g /100 g dw) was similar to the content obtained in leaves of *Cymbopogon citratus* (37.53 ± 0.67%) by [15] but lower than 55% obtained by [16]. The fiber content obtained for *Vernonia amygdalina* leaves (16.97 ± 1.11%) is lower than 25.47% reported by [17].

The total sugars and ascorbic acid contents of these eight leaves and barks respectively ranged from 1.49 ± 0.02 to 22.56 ± 0.59 g /100 g dw and from 55.76 ± 3.15 to 175.71 ± 2.09 µg /100 g dw. The ascorbic acid content of the *Vernonia amygdalina* leaves (118.36 ± 8.37 µg /100 g dw) was higher than the value (52.0 mg/100 g) reported by [18] for *Vernonia amygdalina*.

The protein content of leaves and barks under study varied from 8.38 ± 0.38 to 43.81 ± 3.69 %. The protein level observed in *Cymbopogon citratus*leaves (14.06 ± 0.88 g /100 g dw) was similar to 15.68 ± 0.83 % reported by [19] about *Cymbopogon citratus* leaves. The protein content found in *Vernonia amygdalina* leaves (43.81 ± 3.69%) was higher than 34.56% reported by[14].

The variations observed in the results may be due to the difference in the geographical conditions (location, soil, climate) where the plants were grown and to their strains [20].

Assessment of phytochemicals

The presence of secondary metabolites (alkaloids, saponins, etc.) in the palm wine preservatives may contribute to its biological value. The bioactive components of the different palm wine preservatives under study were presented in table 2.

Table 2. Phytochemicals of some palm wine preservatives

Palm wine Preservatives	Part	Alkaloids (g /100 g dw)	Total phenolics (g /100 g dw)	Total saponins (mg /100 g dw)
Vernonia amygdalina	Leaf	2.59 ± 0.41^{a}	4.24 ± 0.14^{a}	0.57 ± 0.09^{ac}
Cymbopogon citratus	Leaf	1.37 ± 0.22^{b}	3.91 ± 0.14^{b}	0.59 ± 0.01^{a}
Pimenta racemosa	Leaf	1.59 ± 0.83^{b}	4.80 ± 0.24^{c}	1.03 ± 0.03^{b}
Ocimum basilicum	Leaf	3.61 ± 0.60^{c}	2.66 ± 0.12^{d}	0.67 ± 0.04^{c}
Adansonia digitata	Bark	2.05 ± 0.90^{d}	$4.24 \pm 0.31b^{e}$	1.56 ± 0.06^{d}
Adansonia digitata	Leaf	2.64 ± 0.57^{abcd}	3.03 ± 0.26^{d}	0.59 ± 0.05^{ac}
Garcinia lucida	Bark	2.55 ± 0.88^{abcd}	4.70 ± 0.15^{ce}	1.41 ± 0.01^{e}
Garcinia lucida	Leaf	3.15 ± 0.23^{ad}	4.12 ± 0.55^{c}	1.32 ± 0.04^{f}

Means values within a column having differing superscripts are significantly different at $P < 0.05$

The presence of secondary metabolites (alkaloids, saponins, etc.) in the preservatives added to palm wine may contribute to its biological value. The alkaloids content in wine preservatives ranged from 1.37 ± 0.22 to 3.61 ± 0.6 %, *Ocimum basilicum* having the highest alkaloids content and *Cymbopogon citratus* the lowest. Alkaloids are known to be the most essential potent anti-inflammatory agents of naturally occurring products of secondary metabolism [21].

The phenolics content of leaves and barks varied from 2.66 ± 0.12 to 4.80 ± 0.24 %. *Ocimum basilicum* and *Pimenta racemosa* contained respectively the lowest and the highest phenolics content. Phenolic compounds have been reported to exhibit a wide range of biological effects including anti-bacterial, anti-inflammatory and antioxidant properties [22].

The saponins content of leaves and barks under study varied from 0.57 ± 0.09 mg /100 g dw in *Vernonia amygdalina* to 1.88 ± 0.09 mg /100 g dw in *Garcinia lucida* barks. Saponins have been found useful in the formulation of drugs, in food, drinks and beverage industries as foaming agents [23]. In addition, saponins and phenolic compounds have been reported to have antibiotic, antifungal and antiviral activities [24].

Estimation of antinutrients levels

Anti-nutrient factors are compounds which act to reduce nutrients utilization [25]. The antinutrients of the different palm wine preservatives analyzed were presented in table 3.

Table 3. Antinutrients levels of some palm wine preservatives

Palm wine Preservatives	Part	Phytates (g /100 g dw)	Oxalates (g /100 g dw)	Hydrogen cyanide (µg /100 g dw)
Vernonia amygdalina	Leaf	1.52 ± 0.14^{a}	6.48 ± 1.15^{a}	118.36 ± 8.37^{a}
Cymbopogon citratus	Leaf	5.57 ± 0.28^{b}	1.24 ± 0.58^{b}	104.05 ± 2.13^{b}
Pimenta racemosa	Leaf	1.18 ± 0.56^{ad}	1.68 ± 0.01^{b}	153.81 ± 4.26^{c}
Ocimum basilicum	Leaf	4.03 ± 1.71^{be}	2.10 ± 0.59^{b}	87.71 ± 12.83^{d}
Adansonia digitata	Bark	9.76 ± 2.18^{c}	11.08 ± 0.58^{c}	92.61 ± 4.22^{d}
Adansonia digitata	Leaf	0.71 ± 0.14^{d}	1.72 ± 0.01^{b}	55.76 ± 3.15^{e}
Garcinia lucida	Bark	2.79 ± 0.28^{e}	5.72 ± 1.15^{a}	83.41 ± 8.43^{d}
Garcinia lucida	Leaf	3.22 ± 0.01^{f}	12.06 ± 0.01^{d}	175.72 ± 2.09^{e}

Means values within a column having differing superscripts are significantly different at $P < 0.05$

Phytic acid in plants is known for its chelating effect on certain essential mineral elements such as Ca, Mg, Fe and Zn to form insoluble phytate salts [26]. Phytates in leaves and barks analyzed ranged from 0.71 ± 0.14 to

9.76 ± 2.18 %. The lowest level was found in *Adansonia digitata* leaves and the highest in *Adansonia digitata* barks. The phytates content of *Vernonia amygdalina* leaves is quite higher than 1.31 ± 0.03 % observed by [14].

Oxalates can bind to calcium in food thereby rendering calcium unavailable for normal physiological and biochemical roles [27]. The oxalate content in the eight leaves and barks under study ranged from 1.24 ± 0.58 to 12.6 ± 0.01%. The lowest content was found in *Cymbopogon citratus* leaves and the highest in its barks. The oxalates levels observed in *Vernonia amygdalina* leaves is higher than 0.96 % reported by [14]. In the palm wine preservatives under study, the oxalates contents are higher than the permissible level which is of 250 mg/100 g fresh sample[28].

Hydrogen cyanide is an extremely poisonous substance formed by the action of acids on metal cyanides. The levels of hydrogen cyanides ranged from 55.76 ± 3.15 to 175.72 ± 2.09 µg /100 g dw. The lowest level was obtained in *Adansonia digitata* leaves and the highest in *Garcnia lucida* leaves. Large dose of hydrogen cyanide can cause death within few minutes, while smaller dosages may result to stiffness of the throat, chest, palpitation and muscle weakness. The result obtained falls within the threshold value (below 350 mg/ 100g) reported as safety limit [29].

CONCLUSION

The leaves of *Cymbopogon citratus* (Citronella), *Pimenta racemosa* (bay leaf), *Vernonia amygdalina* (bitter leaf), *Ocimum basilicum* (Massep); the barks and the leaves of *Garcinia lucida* (Essok) and of *Adansonia digitata* (baobab) used in palm wine preservation contain nutrients (lipids, proteins, minerals, etc.) and phytochemicals (alkaloids, saponins, phenolics). However, their levels of some anti-nutrients (oxalates) are above the safety limits. Therefore, caution should be paid when using those leaves and barks in palm wine preservation.

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