Effect of Drying Temperatures on Mineral Composition and Bacterial Populations of *Vernonia amygdalina* (Bitter Leaf)

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Abstract
Vernonia amygdalina (bitter leaf) is widely grown and consumed in Nigeria and parts of Africa. Mainly used fresh, it is also dried to improve shelf life. The effect of drying temperatures on the mineral composition and bacterial population of Vernonia amygdalina was studied using standard procedures. Drying temperatures (oven and sun) caused significant reduction ($p<0.05$) in Cu, Na, Mg, and K. However, Zn, Fe, Ca, P, and Mn were not affected by the drying temperatures. The least available nutrient was Cu (0.01±0.0 – 0.013±0.00%) and Zn (0.03±0.00%) and the highest was K ranging between 3.7±0.01 and 3.8±0.01%. The bacterial population was highest ($p\geq0.05$) in fresh samples. Fresh samples had the highest mean total heterotrophic bacterial population of $4.0\pm0.4 \times 10^4$ CFU g^{-1} while the oven dried samples had the least of $2.075 \pm0.6076 \times 10^4$ CFU g^{-1}. Similarly, the coliform counts were 2.350±0.4435 CFU g^{-1}, 1.375±0.2986 CFU g^{-1} and 1.650±0.4041CFU g^{-1} for fresh, oven and sun dried samples respectively. A total of six (6) bacterial genera were identified. The highest occurring was Bacillus spp occurring in all treatments at varying frequencies. Others include Staphylococcus spp, E. coli, Serratia spp, Lactobacillus spp and Pseudomonas spp in order of occurrence. This study proved that drying temperatures did not alter the essential mineral content (Na and K) of the bitter leaf and can be used to prolong its shelf life. The presence of food poisoning bacteria in V. amygdalina in this study poses a public health challenge.

Keywords: *vegetables; bacterial population; mineral composition; Vernonia amygdalina; drying temperature.*

INTRODUCTION

Vernonia amygdalina, commonly known as bitter leaf is the most widely cultivated species of the genus *Vernonia*, with about 1,000 species of shrubs belonging to the family Asteraceae (Munaya, 2013). Traditional vegetative cultivation of the bitter leaf is by stem cutting in most West African countries like Nigeria, Cameroon, Gabon, and Democratic Republic of Congo. Named after an English Botanist, William Vernon (Clarke, 2013), it is also known as ironweed and frequently found in gardens (Schiffers, 2000). With high adaptability to various climes, it has been successfully cultivated in different regions and countries (Ibrahim *et al.*, 2010). True to its name, bitter leaf is bitter to taste but surprisingly delicious in meals (Abosi and Raseroka, 2003). *Vernonia amygdalina* is called Omjunso in East Africa especially Tanzania, Onugbo in Igbo, Orugbo in Yoruba, Etidot by the Efiks and the Ibibios. Other names are Oriwo in Edo, and Chusa-doki shiwaka in Hausa (Agbogidi *et al.*, 2013).

Botanically, bitter leaf is a shrub that grows in tropical Africa, about 2.5m tall with a petiolate leaf (Ijeh *et al*. 2008). The leaves are green with a characteristic odor and bitter taste. However, beyond the bitterness are numerous nutritional and health benefits (Agbogidi *et*

al., 2013; Kubola and Sirianornpun, 2008). The bitter leaves of *V. amygdalina* have been successfully used to alleviate the problems of micronutrients and malnutrition in tropical African countries including Nigeria (Agbogidi *et al.*, 2013; Kubola and Sirianornpun 2008; Ejoh *et al*., 2005). These cost-friendly leaves have been found rich in Beta carotene, vitamin C as well other essential minerals such as iron, phosphorus, calcium and potassium (Agbogidi *et al.,* 2013; Musa *et al.,* 2011). Other important constituents are proteins, ascorbic acid, folic acid as well as dietary anaemia factors (Abosi and Raseroka, 2003). The plant has also been found with significant levels of lipids, carbohydrates, proteins and fiber with essential amino acids (Ejoh *et al.,* 2003).

Traditionally, *V.amygdalina* is used in Nigeria as a green vegetable and spice in soup in the popular bitter leaf soup especially in the southern parts. Its aqueous extract is also used as a therapy mostly in Northern Nigeria (Sabiu and Wudii, 2011). Also in the North, it has been added to horse feed for strength and fat (Farombi and Owoeye, 2011). Elsewhere, it has been used as hops in beer making in Ethiopia (Farombi and Owoeye, 2011; Getahun, 1976). The leaves have been used as a quinine substitute in treating ailments (Farombi and Owoeye, 2011; Masaba 2000).

Medically, the plant has been successfully applied in the treatment of many ailments such as diabetes, malaria, infertility, nausea, liver diseases, kidney diseases, bacterial infections, parasitic infestations as well as diseases of the gastrointestinal tracts (GIT) (Farombi and Owoeye, 2011; Swee *et al*., 2010; Gbolade, 2009; Adedapo *et al.,* 2007; Moundipa *et al*., 2005; Huffman, 2003; Cos *et al.,* 2002; Kambizi and Afolayan, 2001; Anderson *et al.,* 2000; Masaba, 2000; Koshimizu *et al.,* 1994). Etkin, 2002 has recommended the use of bitter leaf stem as chewing stick to maintain good dental health. Its water extract is also useful in treating various plant fungal diseases (Nduagu *et al*., 2008; Ogbebor *et al.,* 2007). Further, the aqueous extracts of *V. amygdalina* have been shown with anti cancer properties (Khalafalla *et al*., 2009; Gresham *et al*., 2008; Howard *et al*., 2006; Izievbigie *et al*., 2004).
With up to 15% of the world's population still without food, there is need to prevent food spoilage (Esper and Mühlbauer, 1998). Drying is the oldest preservation method involving drying, dessication and dehydration of food (Boyer and Huff, 2009; University of California, 2014; Brian, 2002). It involves a reduction in moisture to slow down spoilage organisms and enzymes and thus preserving the food. In Nigeria, vegetables like bitter leaf form a major part of the diet. These are however, seasonal, thus the need to preserve by drying to increase their shelf life and keep the minerals intact (Salle 2008). Drying has been successfully used to extend the shelf life of *V. amygdalina* for local use in Nigeria and more so for export.
Although it remains delicious, it is unknown if the plant retains its important minerals after drying. The aim of this research was to evaluate the effect of different drying temperatures on the mineral composition and bacterial population of *V. amygdalina.*

MATERIALS AND METHODS

Sample Collection

Ten Fresh *Vernonia amygdalina* (bitter leaf) samples were collected from gardens in University of Port Harcourt and its environs in Rivers State, Nigeria. Samples were categorized according to their treatment. All samples were shared into three for various treatments and labeled properly. One part was sun dried for four days, the second part was oven dried at 60℃ for 24 hours and the third part was used immediately for the experiment.

Mineral Analysis

The mineral analysis involved two steps; digestion of sample and mineral determination.

To digest the sample, 2 g of blended sample was added to a digestion tube and 12 ml of Trioxonitrate (v) acid (HNO_3) added. The mixture was left overnight at room temperature. Then 4 ml of Perchloric acid ($HCLO_4$) was added to the mixture and kept in the fumes cupboard and temperature gradually increased from 50 to 300℃ for 85 minutes. Following digestion, the mixture was transferred to a 100 ml volumetric flask and volume completed with distilled water.

Further, mineral determination was done using Atomic Absorption Spectrometry (Alpha 4A AAS), Flame Photometry (Micronal B260) and Visible Spectrometry (Asaolu *et al*., 2012).

Microbiological Analysis

Enumeration of total heterotrophic bacteria and coliform bacteria

Ten (10) grams of *V. amygdalina* sample from the different treatments was weighed, blended and homogenized in 90 ml of sterile normal saline to give a dilution of 1:10. Subsequent serial dilutions were made by adding 1.0 ml to 9.0 ml fresh sterile diluents. Finally, 0.1 ml of appropriate dilutions (10^{-5} and 10^{-6}) was plated out on sterile Plate Count Agar (PCA) and MacConkey Agar (MCA) in triplicates, using spread plate method (Cheesebrough, 2006). All set up were incubated at 37 ^{o}C for 24 h. Plate count agar and MacConkey agar plates which grew colonies between 30 and 300 were counted and recorded in colony forming unit per gram (CFU g^{-1}) of vegetable using the formula: CFU g^{-1} = Ave. colonies×1/vol. plated ×1/dil. factor. Characteristic colonies were subcultured on sterile Nutrient Agar (NA) plates to obtain pure colonies. Pure isolates were identified using morphological and biochemical tests such as Gram's test, motility test, citrate utilization test, Methyl Red – Voges Proskauer (MR-VP) test, Catalase test, Indole Acetic test, Triple Sugar Iron (TSI) test (Cheesebrough, 2006).

DATA ANALYSIS

Data analysis was done using Microsoft excel software. A statistical package (SPSS version 22) was used to analyze the variance between means (ANOVA) at $p \leq 0.05$ and where difference occurred Student Knewman Keul's (S-N-K) test was used to separate the means.

RESULTS

The results of the effect of temperature on the available minerals of *V. amygdalina* as presented in Table 1 showed significant decrease ($p<0.05$) in Cu, Na, Mg, and K. However, other minerals Zn, Fe, Ca, P, and Mn were not affected by the various treatments. The least available nutrient was Cu (0.01±0.0 – 0.013±0.00%) and Zn (0.03±0.00%) and the highest was K ranging between 3.7±0.01 and 3.8±0.01%.

Table 1: Variation of mineral composition of *V. amygdalina* subjected to different drying temperatures

Treatments	Mineral Composition (%)								
	Cu	Zn	Na	Fe	Ca	P	Mg	Mn	K
Fresh (control)	0.013±0.0^{ab}	0.03±0.0^{a}	0.04±0.0^{ab}	0.04±0.01^{a}	0.15±0.01^{a}	0.42±0.01^{a}	0.45±0.02^{a}	0.6±0.02^{a}	3.7±0.03^{a}
Oven dried	0.013±0.0^{b}	0.03±0.0^{a}	0.04±0.0^{b}	0.04±0.01^{a}	0.16±0.01^{a}	0.42±0.01^{a}	0.49±0.015^{b}	0.6±0.01^{a}	3.8±0.01^{b}
Sun dried	0.012±0.0^{a}	0.03±0.0^{a}	0.03±0.0^{a}	0.04±0.01^{a}	0.16±0.01^{a}	0.41±0.01^{a}	0.48±0.02^{ab}	0.6±0.02^{a}	3.7±0.01^{a}

*Means with the same superscript along the columns are not significantly different ($p \geq 0.05$)

Results of drying temperatures on total heterotrophic bacterial population and coliform counts of *Vernonia amygdalina* subjected to various treatments showed significant differences ($p \leq 0.05$) (Table 2)

Table 2: ANOVA Table showing level of significance ($p \leq 0.05$) between the parameters tested for the different treatments

		Sum of Squares	df	Mean Square	F	Sig.
Total heterotrophic bacteria (x10^{4}cfu g^{-1}) * Treatments	Between Groups (Combined)	9.172	2	4.586	16.812	.001
	Within Groups	2.455	9	.273		
	Total	11.627	11			
Coliform counts (x10^4 cfu g^{-1}) * Treatments	Between Groups (Combined)	2.022	2	1.011	6.751	.016
	Within Groups	1.347	9	.150		
	Total	3.369	11			

The bacterial population was higher ($p \geq 0.05$) in fresh than the oven dried and sundried samples (Table 3). The fresh had the highest mean total heterotrophic bacterial population of 4.0±0.4 × 10^4 CFU g^{-1} while the oven dried and Sun dried had the least with 2.075 ±0.6076 × 10^4 CFU g^{-1} and 2.225±0.5058 × 10^4 CFU g^{-1} respectively. The same trend was also observed for coliform counts in the different treatments.

Table 3: Effects of drying temperatures on total heterotrophic bacterial population and coliform count of *Vernonia amygdalina* subjected to various treatments

Treatments	Bacterial population (x10^{4}cfu/g)	
	Total heterotrophs	Coliforms
Fresh (Control)	4.000±0.4397^b	2.350±0.4435^b
Oven dried	2.075±0.6076^a	1.375±0.2986^a
Sun dried	2.225±0.5058^a	1.650±0.4041^a

*Means with the same superscript along the columns are not significantly different ($p \geq 0.05$)

Coliform count was highest in fresh samples with a mean of 2.350±0.4435 x 10^4 CFU g^{-1} and least in the oven – dried samples with a mean of 1.375±0.2986 x 10^4. CFU g^{-1}

A total of six (6) bacterial genera were isolated and identified (Fig. 1). The highest occurring was *Bacillus* spp (100%). This was closely followed by *Staphylococcus* spp (60%) which appeared in the fresh samples. Other genera included *E. coli*, *Serratia* spp, *Lactobacillus* spp and *Pseudomonas* spp in order of occurrence. All isolated bacteria were most abundant in the fresh samples and least in the oven dried (Fig 1). However, *E. coli*, *Serratia* spp., *Lactobacillus* spp., *Staphylococcus* spp. and *Pseudomonas* spp. failed to grow in oven dried samples, with only *Lactobacillus* spp. failing to grow in sun dried samples

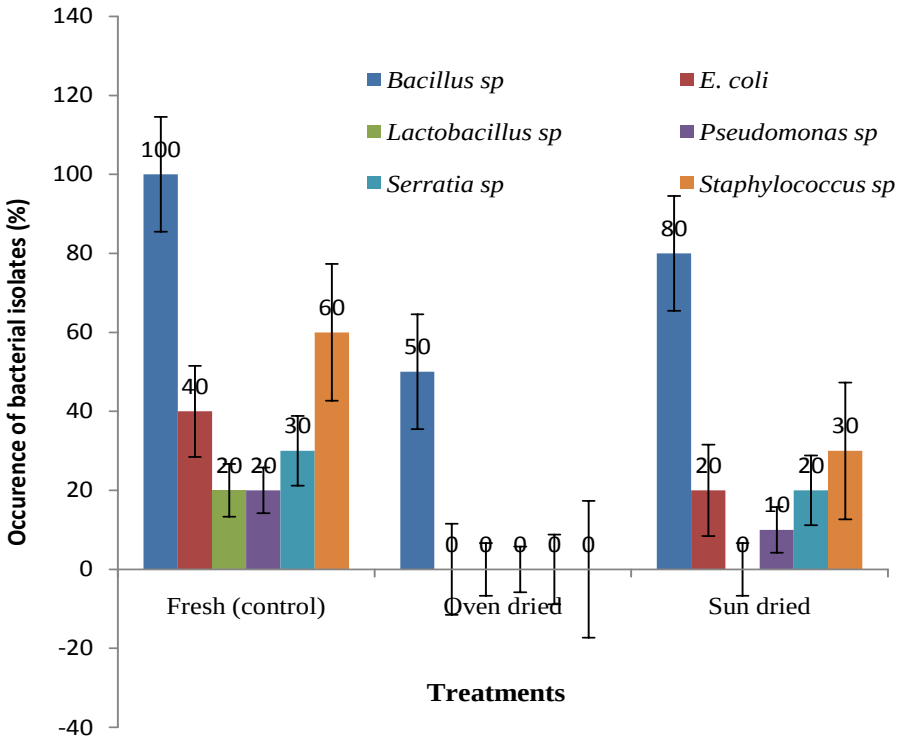

Fig.1: Percentage occurrence of bacterial isolates in *Vernonia amygdalina* samples subjected to different drying temperatures

DISCUSSION

The rich mineral composition of *V. amygdalina* as in our study, confirms the plant as rich source of most essential minerals (Musa *et al.* 2011). The mineral composition is indicated in this progression K>Mn>Mg>P>Ca>Fe>Na>Zn>Cu. It was observed that mineral elements for the different treatments were significant at $P<0.05$ level with oven dried treatment having the highest value for potassium (3.80g/100g). There was little decrease in the same mineral element with sun dried treatment (3.7g/100g). This is as previously reported by Muhammed *et al*. (2014). While Potassium was the predominant mineral analyzed, Cu was least. The mineral composition of bitter leaf is enough to meet the Recommended Daily Allowance per Adult. The minimum potassium requirement as set by the 1989 RDA is 2000 mg daily.
Sodium and potassium are important intracellular and extracellular cations respectively (Dresser *et al*., 2001). A study by Adrogué and Madias (2014) indicate that sodium and potassium are important in homeostatic control of hypertension. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Akpanyung, 2005).

The composition of magnesium ranged from 0.45 g100 g^{-1} to 0.49 g100 g^{-1} for fresh and dehydrated bitter leaves respectively. The result obtained meets the RDA of 400 mg day^{-1} for men 19-30 years old and 310 mg day^{-1} for women 19-39 years old (Asaolu *et al*., 2012). The iron content is lower than the RDA requirement. Iron is an important trace element in the human body (Abbaspour *et al.*, 2014). It is important in many physiological roles such as oxygen transport, DNA synthesis as well as electron transport, infection control and cell mediated immunity. The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide (Abbaspour *et al.*, 2014).
The levels of zinc obtained in this research are similar to those reported by Asaolu *et al.* (2012). Zinc is essential micronutrient for human growth and immune functions (Bhowmik, and Chiranjib, 2010). The level of phosphorus obtained showed moderate concentration. Calcium and phosphorus are essential nutrients in humans (Dorozhkin and Epple, 2002). They are significant in the bones and urinary system. The drying temperatures did not alter the mineral composition of *V. amygdalina*, hence its consumption will make up for the recommended daily allowance (RDA).

Drying temperatures had significant effect on the bacterial population of *V. amygdalina* . This could be because most of the bacteria isolated lacked heat-stable enzymes and protein synthesis that function properly at high temperatures (Joanne *et al.*, 2014). Drying process involves the removal of moisture to inhibit the activities of food spoilage microorganisms. It also minimizes enzyme-mediated deteriorative reactions, thus increasing the food shelf life (Doymaz, 2005). The bacterial population in *V. amygdalina may* be due to many factors, such as growth environment, handling, exposure during sun drying and other processing factors.
The increased mean total heterotrophic bacterial and coliform count in fresh *V. amygdalina when* compared to the dried samples as observed in this study may be due to the presence of growth factors such as moisture. Lochhead and Chase (1943) explained that moisture is required for the proliferation of microorganisms. Although the moisture content of *V. amygdalina* was not analysed in this study, Udochukwu *et al.* (2015) in their study recorded high moisture content (86.2%) in fresh *V. amygdalina* which further explains the increase in the bacterial populations. The decrease, therefore, in bacterial population in dried samples irrespective of contributory environmental factors supports drying as a good method for preserving *V. amygdalina.* The marked reduction in the bacterial population with increased

temperature had been reported by other researchers (Joanne *et al*., 2014; Lochhead and Chase, 1943).

All six (6) genera of bacteria (*Bacillus* spp., *Escherichia coli, Lactobacillus* spp., *Pseudomonas* spp., *Serratia* spp. and *Staphylococcus aureus*) were isolated from fresh samples. The presence of coliform bacteria from all treatments is disturbing and may indicate fecal contamination. This could be from handlers and other environmental factors (Harwood *et al*., 2000). *Bacillus* spp. occurred most in all treatments of *V. amygdalina*. This organism is a normal flora of bitter leaf plant. They also produce spores, thus enabling them survive harsh environments. The spore forming ability of these organisms may explain their presence in dried samples. Spore formation is an adaptive feature of organisms for adverse conditions such as temperature (Peberdy 1980, Young and Fitz-James, 1959). These organisms have been associated with food poisoning. *Bacillus cereus* is known to produce enterotoxins previously implicated in cases of food poisoning (Dierick *et al.* 2005; Terranova and Blake 1978). Cases of *B. cereus* food poisoning may be mild, but have been fatal in some cases (Dierick *et al.* 2005). Similarly, organisms such as *E. coli* and *S. aureus* are normal flora of man. Their presence suggests poor handling practices leading to contamination (Nwankwo and Akani, 2017). The isolation of only *Bacillus* spp in oven dried samples further confirms this explanation, as most bacteria would find 60^{o} C an unfavorable temperature for their growth and survival. This is in agreement with the works Joanne *et al*., (2014) and Abosede and Lawrence (2013).

Some of these microorganisms found on bitter leaf are associated with food poisoning. They produce toxins known as enterotoxins resulting in vomiting, nausea and diarrhea. These organisms include; *Staphylococcus aureus, Escherichia coli* and *Bacillus* sp. Numerous cases of food poisoning associated with consumption of freshly washed bitter leaf may be explained by this finding. The present study revealed that these organisms were present mostly in fresh samples. The presence of these food poisoning organisms is a public health risk. There is need for caution in preparation and consumption of this vegetable, especially when fresh.
Microbial contamination could be reduced and prevented if the bitter leaf is processed, packaged and stored aseptically. Maintenance of strict hygiene practices during processing and drying is important to produce dehydrated vegetables with good microbial quality and extended shelf life. This would ensure public health safety. The high mineral content of *V. amygdalina* makes it as a good supplement and it could augment dietary deficiencies.

CONCLUSION

The essential minerals in *V. amygdalina* were not reduced by drying temperatures. Drying is therefore a good method of preservation for the vegetable. This will improve the shelf life and make for export to improve reach. It therefore makes the vegetable a source of foreign exchange.
Similarly, the bacterial load was reduced remarkably by drying. Again, this supports drying as a good preservation method. The presence of food poisoning bacteria is reduced by drying. There is need for caution in consuming fresh bitter leaf. Food poisoning bacteria constitute public health hazard. This should be considered in preparation.
This vegetable can be used as a good source of essential minerals like potassium, calcium, phosphorus, etc.

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