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RESEARCH ARTICLE

VERNONIA AMYGDALINA LEAVES MARKETED IN BENIN CITY: ASPECTS OF SELECTED MINERAL ELEMENTS AND VITAMINS PROFILING AND INFLUENCE OF PROCESSING AND STORAGE STUDIES

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ABSTRACT

Vernonia amygdalina Leaves is a common additive used for diet and ethnomedicinal purposes. This work entailed the investigations of *Vernonia amygdalina* Leaves for its levels of copper (Cu), cobalt (Co), zinc (Zn), iron (Fe), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chromium (Cr) and selenium Se) levels, as well as the levels of ascorbic acid, thiamine, riboflavin and pantothenic acid. Also, the study investigated the responses of the studied vitamins to processing and storage. All investigations were carried out using standard methods. Samples were stored in the open laboratory and at water activities (a_w) of 0.23, 0.52 and 0.97. Duration of storage was two months. Findings indicated that the ranges of occurrence of the examined mineral elements were K: (8,905.987±39.374 -11,698.115±25.293) mg/kg; Ca: (750.627±31.279 - 965.987±29.169) mg/kg; Mg: (322.607±7.389 - 423.650±26.115) mg/kg; Na: (21.927±3.843 to39.308±3.572) mg/kg; Zn: (14.258±2.297 - 26.403±1.712) mg/kg; Fe: (9.914±1.298 - 27.580±4.267) mg/kg; Cu: (1.917±0.186 -5.143±0.257) mg/kg; Cr: (1.283±0.317 - 3.378±0.422) mg/kg; Se: (0.310±0.062 - 0.394±0.085) mg/kg and Co: (0.145±0.018 - 0.241±0.098) mg/kg. Additionally, the values obtained for the examined vitamins were: ascorbic acid (14.948±0.188 - 24.849±0.739) ppm; thiamine (2.278±0.174-3.284±0.069) ppm; riboflavin (3.285±0.491 - 4.685±0.277) ppm and pantothenic acid (2.481±0.152 - 2.481±0.152) ppm .Results further revealed that processing and storage negatively affected the values of the studied vitamins. Statistical analysis P<0.05 revealed significant differences between the values of the investigated mineral elements, as well as those of the vitamins. Significant differences (P<0.05) were also indicated among the processing and storage values obtained for the examined vitamins in the processed and stored *Vernonia amygdalina* leaves. Hopefully, these findings will be useful in policy formulation of standard methods for handling of *Vernonia amygdalina* leaves.

KEYWORDS

Vernonia amygdalina, phytochemicals, water activity (a_w), storage.

1. INTRODUCTION

Vernonia amygdalina leaves which are wide used as additive in many diets and ethnomedicinal remedies preparations are known by variety of local names in Nigeria. Remarkably, noted that the Igbos call it Onugbu, the Ibiobios call it Etidot, in Youruba it is called Ewuro, Ityuna among the Tiv), Oriwo among the Bini and Chusardoki or Fatefate in Hausa Language (Kokwaro, 2009). Furthermore, reported that in Uganda, *Vernonia amygdalina* is called Mululiza or mubirizi, Ebichaa in Ethiopia and Awonwono in Ghana (Kiguba et al., 2016). *Vernonia amygdalina* is a perennial shrub and belongs to the Asteraceae family (Sunmonu et al., 2022).

Vernonia amygdalina leaves are known for the bitter taste they introduce to diets when incorporated as additive in the preparation of such diets. With respect to their medicinal values, these authors: reported various medicinal relevance of *Vernonia amygdalina* (Igile et al., 1995; Udochukwu et al., 2015; Ojimelukwe and Amaechi, 2019; Okunlola et al., 2019). Also, the Medical Traditional Healer Association in Rukararwe, Uganda as remarked by Njan et al. (2008) used *Vernonia amygdalina* to produce greenish powder packed in sachet and consumed as tea by patients suffering from malaria. Additionally, there are remarkable industrial utilizations of *Vernonia amygdalina* leaves. For instance, Dibie and Dibie (2024) posited that some fish farmers in Nigeria use liquid extracts generated from mashed and squeezed fresh *Vernonia amygdalina* leaves as antibacterial agent in their culture farms. Furthermore, reported that *Vernonia amygdalina* leaves have been used in place of hops in the beer-brewing sector (Adama et al., 2011). Also, reported that the honey wine called Tei in Ethiopia is produced from bitter leaf (Yeap et al., 2010). Despite the numerous benefits linked to the use of *Vernonia amygdalina*, there is need for caution. In particular, reported that the use of *Vernonia amygdalina* is dose–dependent for successful results (Ibrahim et al., 2009). It is imperative therefore, that the phytoconstituents of *Vernonia amygdalina* should not only be investigated routinely because of changes in methods of cultivation and environmental factors that could affect them, but the responses of the Phytoconstituents in *Vernonia amygdalina* to factors that could affect their levels of occurrence postharvest, should also be investigated and documented. Hence this study was initiated.

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The interest of this study in part, was to investigate *Vernonia amygdalina* leaves samples obtained from different locations in Benin City for their copper (Cu), cobalt (Co), zinc (Zn), iron (Fe), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chromium (Cr) and selenium Se) levels; the levels of ascorbic acid, thiamine, riboflavin and pantothenic acid contained in them. Another portion of the work was concerned with the study of the responses of the investigated vitamins to processing and storage. The quality indices investigated in this work indicate bio-activities that are nutritional and therapeutically relevance. Processing methods investigated were sun drying and grinding. Though the use of sun drying as a method for dehydration of *Vernonia amygdalina* leaves to enhance their shelve lives is practiced by some handlers of *Vernonia amygdalina* leaves, the method as commonly practiced is highly unstandardized. Considerations from the point of view of safety and quality, dictate that foods processing and handling methods should be standardized. Findings from this study even if partly, will be relevant in the formulation of standard sun drying method for processing of *Vernonia amygdalina* leaves. It is imperative to mention that literature reports on the responses of the studied vitamins constituents of *Vernonia amygdalina* leaves to sun drying and grinding are scarce. Hence findings from this work will help expand this frontier of knowledge

The storage aspects of this work entailed storage of samples in both closed and opened containers and kept in the open laboratory. Furthermore, samples were stored at water activity (aw) of 0.23, 0.052 and 0.97. Literature reports on the influence of storage on the compositional chemistry of *Vernonia amygdalina* leaves appeared difficult to get. In particular, there is dearth of information on the influence of aw on the compositional chemistry of *Vernonia amygdalina* leaves. Findings from this study will fill the identified gap in knowledge even if partly.

It should be emphasized that food safety, stability and other properties as shown by the works of these researchers viz: are more accurately predicted from aw than from water content (Acker 1969; Schoebel et al., 1969; Labuza et al., 1970; Lajollo et al., 1971; Eichner and Karel, 1972; Ukhun, 1984; Ukhun, 1986; Ukhun and Uwatse, 1988; Ukhun and Dibie, 1989; Ukhun and Dibie, 1991; Dibie and Ukhun, 2019a; Dibie and Ukhun, 2019b; Dibie and Ukhun, 2019c; Dibie and Ukhun, 2020a; Dibie and Ukhun, 2020b; Dibie and Dibie, 2024). It is remarked by Dibie (2019) that aw is a more modern concept than moisture content in food chemistry. Also, reported that the concept of water activity is nowadays universally adopted by food scientists and technologists to quantify water availability (Coultate, 2002). Additionally, remarked that the storage quality of food does not depend on the water content, but on water activity (aw) (Belitz et al., 2009). These are reports that make water activity studies significant in foods stability investigations.

2. Materials and Methods

2.1 Sample Collection

Fresh *Vernonia amygdalina* leaves used in this study were obtained from the following open markets in Benin City:- Uselu, New Benin, Ogida, Jeromi, Oliha, Ewosha, Okah, Useh, Ekae, Oluku, Agbado, and Egor markets. In each of the markets, five samples (purchased from five different sellers) of fresh *Vernonia amygdalina* leaves were procured. The leaves were identified by Prof. Akinnibosun Henry Adewale a taxonomist in the Department of Plant Biology and Biotechnology (PBB) of University of Benin, Benin City and voucher number UBH-V342 was assigned to the *Vernonia amygdalina* leaves.

2.2 Samples Inspection and Cl0eaning

Samples inspection and cleaning were carried out in accordance with the method described by (Dibie and Ukhun, 2022). The method consisted of inspection, identification and removal of foreign materials including unwanted plants and/ or parts of plants.

2.3 Samples Preparation

Fresh *Vernonia amygdalina* leaves were sun dried to constant weight. This gave the sun dried samples. Thereafter, Black and Decker 650W, BX550 blender was used to grind the sun dried samples, after which the ground samples were sieved using a 16 – mesh standard sieve (Pascall Eng. Co. Ltd. Sussex, England).

2.4 Samples Storage

Three hundred grams of sun dried and ground samples were weighed in triplicates and put into different 500ml glass beakers (Pyrex glass). Thereafter, the various 500ml glass beakers containing the weighed samples were stored in air tight desiccators wherein aw of 0.23, 0.52 and 0.97 were established in accordance with the method prescribed by (Rockland, 1960). Samples storage duration was 2 months and on monthly basis they were examined for the studied parameters. All the storage desiccators were placed on laboratory bench and at ambient conditions.

2.5 Determinations of Selected Vitamins

2.5.1 Determination of pantothenic acid using HPLC

2.5.1.1 Extraction

Extraction of pantothenic acid from the different samples was carried out in an adaption of the method described by (Ciulu et al., 2013). The procedure entailed the following:-

Two grams of finely ground sample was weighed into a 25ml conical flask. This was followed by the addition of 10ml of 8% (v/v) aqueous solution of trichloroacetic acid. Subsequently, with the aid of magnetic stirrer, the contents of the 25ml conical flask were mixed for 10 min and thereafter, centrifuged at 2000rpm for 5 min to separate the proteins. The supernatant solution was filtered using Whatman No. 41 filter paper into a 20ml volumetric flask and made up to the 20ml mark with type 1 ultrapure water. This gave the sample solution.

2.5.1.2 Standard preparation

The method described was used to prepare the stock standard solution by (AOAC, 1975). The procedure consisted of the following:-

Ten milligrams of D-Pantothenic acid hemicalcium salt was weighed and put into a 100ml volumetric flask, after which 4ml of 0.002M NaOH was added. Thereafter, the volumetric flask was shak0en until the D-Pantothenic acid dissolved completely. Subsequently, the solution was made up to 100ml with 0.001M sodium dihydrogen phosphate solution (pH = 5.5). The standard solution was prepared daily and stored in the dark at 40C.

2.5.1.3 Sample cleanup for Quantitative determination of Pantothenic Acid

The sample clean up in preparation for quantitative determination of pantothenic acid using HPLC was done by passing some of the sample solution through a Waters 0.2µm nylon filter.

2.5.1.4 Quantific0ation of Pantothenic Acid

The HPLC equipment used for quantitative determination of pantothenic acid is composed of a series HP1000 quaternary pump, fitted with an HP 500 uv-vis absorption detector product of Hewlett – Packard (Waldbronn, Germany), and a 20µl Rheodyn injection loop (Rohnert Park, CA). They were controlled by Hewlett – Packard HPCHEM software. The column used was a 250 × 4.00mm (ID) K0romasil 100C18 column packed with 5-µm particles (Teknokroma, Barcelona, Spain). The column was thermostatted at 200C, with a Mod. 8792 column thermostatting system, product of Spectra – Physics (San Jose, CA): the mobile phase was 0.025% trifluoroacetic acid (v/v in water), delivered at a flow rate of 1.0ml/min. The pantothenic acid concentration determination was based on ultraviolet light absorption at 210nm. Injection volume was 40µl. Peaks identifications were by comparison of their retention times with those of standards and the various peaks areas were determined with the aid of Jasco software. Triplicate determinations were carried out for each sample.

2.5.2 Ascorbic Acid Determination with HPLC.

2.5.2.1 Extraction

The methodas prescribed was used to carry out the extraction of ascorbic acid (AOAC, 1975; Watada, 1982). Solvent used for the extraction consisted of 6% meta phosphoric acid containing 1×10-6M EDTA and 1×10-7M diethylthiocarbamate. Method of extraction used is described below:-

Two grams of ground sample was put into a 250ml conical flask protected from light. After which 10ml of the extracting solvent mixture that is 6% meta phosphoric acid, 1×10-6M EDTA and diethylthiocarbamate [18:1:1] was added. The addition of the solvent mixture was accompanied by constant but slow bubbling of nitrogen gas into the flask, during which the flask was gently stirred using magnetic stirrer for 30mins. Thereafter, the flask was left to stand for 1min and subsequently, the plant – HPO3, EDTA and diethylthiocarbamate macerate mixture was filtered using Whatman No 1 filter paper into a 100ml volumetric flask, protected from light. Nitrogen gas was bubbled into the volumetric flask after which it was stoppered. The processes of extraction and filtration were repeated three times; and in each time, 10ml of the extracting solvent mixture was used. The mixture in the volumetric flask was made up to the 100ml mark with purified water obtained from Milli-Q system. This gave the sample solution. Sample clean up in readiness for the quantitative determination of ascorbic acid using HPLC was done by passing portion of the sample

solution through a Waters 0.2-µm nylon filter. 5ml aliquots of standard and blank solutions were also taken through the entire sample preparation steps.

2.5.2.1 Quantification of Ascorbic Acid

Quantitative determination of ascorbic acid levels in the prepared sample was done using an HP1000quaternary pump, equipped with an HP500 uv-vis absorption detector product of Hewlett-Packard (Waldbronn, Germany), and a 20µl Rheodyn injection loop (Rohnert Park, CA); all controlled by Hewlett – Packard HPCHEM software. The column used was a 250 × 4.00mm (ID) Kromasil 100C18 column that was parked with 5-µm particles (Teknokroma, Barcelona, Spain). The column temperature was maintained at 250C, using a Mod. 8792 column thermostatting system, product of Spectra – Physics (San Jose, CA) and the mobile phase was 1.5% NH4 H2PO4, pH3, that was delivered at a flow rate of 4ml/min. The ascorbic acid level was based on ultraviolet light absorption at 254nm. Injection volume was 20µl. Peaks identification were done by comparing their retention times with those of the standards; and the peak areas were measured using Jasco software. Each sample was prepared and injected in triplicate

2.6 Determination of Thiamine and Riboflavin by HPLC

2.6.1 Reagents and Standards.

High – performance liquid chromatography grade methanol, hydrochloric acid and ammonium acetate that were used in thiswork were products of Merck, (Darmstadt, Germany). Thiamine hydrochloride, thiamine pyrophosphate, riboflavin and potassium hexacyano ferrate (iii) used were products of Sigma – Aldrich (St. Lousi, Mo. U. S. A), anhydrous sodium acetate, glacial acetate, absolute ethanol and sodium hydroxide were products of Panreac (Barcelona, Spain). All reagents used were of analytical grade. Deionized distilled water was obtained with a Milli – Q system (Millipore, U. S. A). The Clara – diastase (from a lot with a stated activity of 53 µ/mg) used was product of Fluka, (Buchs, SwitzerLand).

Stock solutions of thiamine in 20ml/100ml ethanol (pH 3.5 – 4.3) and riboflavin in 20 ml/L acetic acid were stored in the dark at 40C. Thereafter, working solutions were prepared daily from the stock solutions by appropriate dilution (Mondragon-Portocarrero et al., 2011)

2.6.2 Extraction

The extraction method used was a re-modified version of the procedure described by Sanchez-Machado et al. (2004) as reported by (Mondragon-Portocarrero et al., 2011). Homogenization of Sample was initially homogenized. Thereafter, 10g sample was weighed and put into a250ml amber – coloured flask. Subsequently, 50ml of 0.1M HCl was added. This was followed by the immersion of the flask in a shaking water bath maintained at 1000C for 30 min. The flask with its content was thereafter cooled to room temperature. Then the pH of the solution was adjusted to pH of 4.5 by the addition of 2.5M sodium acetate. After which 10mg of Clara – diastase was added and the flask was subsequently shaken. Thereafter the mixture was incubated in an oven maintained at 500C for 3h, with magnetic stirring. At the completion of the incubation, the mixture was allowed to cool to room temperature, following which the mixture was transferred to a 100ml volumetric flask and made to mark with the purified water obtained from Milli- Q system. Subsequently, the mixture was filtered with the aid of a fiberglass filter (No. 8. Schleicher and Schuell, Germany) to obtain the sample solution.

2.6.3 Sample Clean-up for Quantitative Determination of Riboflavin

The sample clean up in preparation for quantitative determination of riboflavin using HPLC was carried out by passing portion of the sample solution through a waters 0.2-µm nylon filter.

Further Treatment of Sample Solution for Thiamine Quantitation

Derivatization of the thiamine content of the sample solution to its fluorescent derivative (thiochrome) was initially carried out. This was done in accordance with the method described by (Mondragon-Portocarrero et al., 2011). The method consisted of the following: Five milliliters of the filtered extract of sample solution was measured into a 10ml volumetric flask. Thereafter, 2.5ml of 0.375M sodium hydroxide containing 1% potassium hexacyanoferrate (iii) was added. After which the mixture was stirred for 5s and left to stand for 60s. This was followed by the addition of 0.25ml of 3.75M HCl and the content of the 10ml volumetric flask subsequently shaken thoroughly to mix.

Prior to HPLC determination, the samples were cleaned by loading them onto a waters C18 Sep – Pak column, which was initially activated by successive passage of 5ml of methanol, followed by 5ml of 0.005M ammonium acetate. 5ml of sample solution was added. Likely interferences were removed by passage of 5ml of a 95: 5 (v/v) mixture of 0.005M ammonium acetate (pH 6.0) and methanol. Thereafter, the vitamins were eluted with 5ml of a 60: 40 (v/v) mixture of 0.005M ammonium acetate and methanol. After which the eluate was passed through a Waters 0.2µm nylon filter prior to HPLC. 5ml aliquots of standard and blank solutions were taken through the sample preparation procedure.

2.6.4 Quantification of Thiamine and Riboflavin

The quantifications of thiamine and riboflavin in the prepared sample were carried out using an HP1000 quaternary pump, fitted with an HP1000 fluorescence detector product of Hewlett-Packard (Waldbronn, Germany), and a 20µl Rheodyn injection loop (Rohnert park; CA); that were all controlled by Hewlett – Packard HPCHEM software. Also fitted was a 250 × 4.6mm (ID) kromasil 100 C18 column that was packed with 5-µm particles (Teknokroma, Barcelona, Spain). The column temperature was maintained at 350C, with the aid of a Mod. 8792 column thermostating system product of Spectra- Physics (San Jose, CA); the mobile phase was a 72 : 28 (v/v) mixture of 0.005M ammonium acetate (pH 6.0) and methanol, that was delivered at a flow rate of 1.2ml/min. The excitation/emission wavelengths are 366/435 for thiochrome and 440/510 for riboflavin. The peaks were identified by comparison of their retention times with those of the standards, after which the peaks areas were determined using Jasco software. For each of the samples, triplicate determinations were done.

2.7 Determination of Selected Mineral Elements Content.

The determinations of the levels of Cu, Co, Zn, Fe, Ca, Mg, Cr, and Se contained in the sample digests were carried out using atomic absorption spectrophotometer. Conversely, the determinations of the levels of Na and K in the sample digests were done by flame photometry.

2.7.1 Digestion of Sample

Wet digestion method as described by Estefan et al. (2013) was used to carry out the digestion of the sample. The method consisted of the use of concentrated tri-acid (HNO3-H2SO4-HClO4) as digestion mixture. The tri-acid mixture (HNO-H2SO4-HCLO4) used was made up of concentrated HNO3, H2SO4 and HClO4 mixed in 10:1:4 ratio (Estefan et al., 2013) and thereafter, allowed to cool. The digestion process commenced with an initial weighing of 2g dried and ground sample into a 300ml digestion flask. After which 10ml of conc. H2SO4 was added, followed by careful swirling of the digestion flask. This was followed by heating of the flask on a thermostatically controlled Gallenkamp hot plate. The temperature of the hot plate was gradually increased to 1450C, after which the heat was maintained at this temperature for 1 hour. This was followed by the addition of 10ml of the tri-acid mixtures (HNO3–H2SO4–HClO4) and subsequently, the temperature setting was gradually adjusted until the temperature was raised to 2400C. Heating at the latter temperature of 2400C was carried out for another 1 hour. It was observed that a clear solution was obtained. The digestion flask and the content contained therein were subsequently allowed to cool to room temperature. This was followed by the filtration of the digest using Whatman No. 42 filter paper. Thereafter, the filtrate was made up to 100ml in a glass volumetric flask with deionised distilled water. Preparation of reagent blank (no plant material) that was subjected to similar digestion, filtration and volume make up with deionised distilled treatment as done with the sample was also carried out.

2.7.2 Measurements

The atomic absorption spectrophotometer (Buck Scientific Model 210 VGP) and flame photometer (Sherwood model 410) used were operated in accordance with the guidelines provided for the equipment. Suitable standards of concentration 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20mg/l (ppm) were prepared and run. Thereafter, their absorbance values were used to construct the respective standard curves. This was followed by the determinations of Cu, Co, Zn, Fe, Ca, Mg, Cr, and Se levels in the respective filtered and diluted digests with the atomic absorption spectrophotometer. On the other hand, the levels of Na and K in the various filtered and diluted digests were determined using flame photometer.

2.8 Statistical Analysis

All determinations done in this study were carried out in triplicates. The data generated from the various investigations were used to calculate the arithmetic means and standard deviations, after which the results were presented as mean ± SEM. Furthermore, with respect to the raw, sun dried, ground and stored samples, data obtained for the for the studied parameters as well as the data generated for the mineral elements levels investigations, were analyzed for statistical significance by two way ANOVA using International Business Machine (IBM) Statistical Package for

Social Sciences (SPSS). Differences were considered significance at P<0.05.

3. Results and Discussion

3.1 Results for Selected Mineral Elements Determinations

The results for the investigations of Cu, Co, Zn, Fe, Na, K, Ca, Mg,Cr and Se levels in *Vernonia amygdalina* leaves obtained from some markets in Benin City are presented in Table 1 below

Table 1: Selected Minerals Elements Levels (mg/kg Dry wt Basis) in *Vernonia amygdalina* leaves obtained from some markets in Benin City

S/N	Sample source	Mineral elements									
	(Markets)	Cu	Co	Zn	Fe	Na	K	Ca	Mg	Cr	Se
1	New Benin	4.677± 0.103	0.226± 0.003	20.767± 1.759	15.933± 0.404	39.308± 3.572	11,698.115± 25.293	752.953± 19.293	328.773± 27.806	2.443± 0.381	0.367± 0.112
2	Ogida	3.357± 0.148	0.241± 0.098	26.403± 1.712	9.914± 1.298	28.933± 2.998	10,196.485± 14.779	907.618 ± 15.249	369.960± 11.489	2.380± 0.165	0.333± 0.135
3	Oliha	1.917± 0.186	0.178± 0.072	17.013± 1.013	13.410± 2.855	21.927± 3.843	9,856.583± 22.410	775.493± 18.389	329.947± 18.076	2.890± 0.185	0.357± 0.139
4	Ewosha	2.947± 0,534	0.218± 0.092	20.787± 3.411	14.107± 1.313	25.541± 3.185	8,914.370± 43.816	883.457± 25.782	330.813± 14.332	1.283± 0.317	0.310± 0.062
5	Okah	2.300± 0.164	0.205± 0.018	19.317± 5.016	19.490± 3.497	33.840± 3.539	9,468.527± 28.631	750.627± 31.279	322.607± 7.389	2.697± 0.417	0.385± 0.079
6	Useh	5.143± 0.257	0.145± 0.018	20.348± 2.412	24.541± 1.065	29.204± 1.593	10,374.210± 27.946	877.135± 17.438	341.185± 13.149	3.378± 0.422	0.326± 0.038
7	Uselu	4.296± 0.286	0.181± 0.049	19.083± 4.171	15.043± 2.146	37.017± 8.152	10,374.295± 35.079	860.105± 12.438	356.940± 10.598	3.362± 0.529	0.393± 0.142
8	Ekae	4.533± 0.370	0.187± 0.059	20.310± 2.675	27.580± 4.267	34.583± 5.627	9,556.342± 20.711	765.673± 9.596	377.805± 22.499	2.473± 0.572	0.394± 0.085
9	Oluku	4.396± 0.287	0.202± 0.019	25.148± 2.159	16.627± 1.978	22.683± 3.528	10,477.820± 30.749	908.557± 14.368	326.690± 25.459	2.592± 0.184	0.337± 0.048
10	Jeromi	4.972± 0.095	0.163± 0.059	17.073± 1.826	13.950± 1.139	31.753± 6.198	10,688.057± 54.416	859.738± 32.671	407.023± 8.334	1.970± 0.474	0.340± 0,068
11	Agbado	2.917± 0.329	0.158± 0.067	14.258± 2.297	15.243± 3.679	29.973± 4.863	8,905.987± 39.374	890.988± 21.429	379.630± 13.929	2.553± 0.106	0.230± 0.069
12	Egor	2.583± 0.379	0.174± 0.086	19.443± 4.852	18.980± 1.850	26.973± 2.498	9,960.170± 43.610	965.987± 29.169	423.650± 26.115	2.903± 0.529	0.338± 0.061

It is discernible from findings that the examined mineral elements occurred in different amounts in the *Vernonia amygdalina* leaves. Notably, as arranged in decreasing order, the range of occurrence of the examined mineral elements were K: (8,905.987±39.374 to11,698.115±25.293) mg/kg; Ca: (750.627±31.279 to 965.987±29.169) mg/kg; Mg: (322.607±7.389 to423.650±26.115) mg/kg; Na: (21.927±3.843 to39.308±3.572) mg/kg; Zn: (14.258±2.297 to26.403±1.712) mg/kg; Fe: (9.914±1.298 to27.580±4.267) mg/kg; Cu: (1.917±0.186 to5.143±0.257) mg/kg; Cr: (1.283±0.317 to 3.378±0.422) mg/kg; Se: (0.310±0.062 to 0.394±0.085) mg/kg and Co: (0.145±0.018 to0.241±0.098) mg/kg. As results indicated potassium occurred most among the mineral elements investigated in the samples. On the other hand, findings revealed that selenium was the least occurring. It is nutritionally desirable that the investigated mineral elements were present in *Vernonia amygdalina* leaves. Assuming bioavailability it is an indication that the examined plant part could be dietary source for the investigated mineral elements.

The noted differences in the levels of occurrence of the examined mineral elements in samples of *Vernonia amygdalina* leaves studied in this work are attributed to varying agricultural practices used to cultivate them; particularly, the chemical composition and quantity of fertilizers applied to them during cultivation. Furthermore, the composition of the soil on which they were cultivated , maturity at the time of harvest, environmental factors, species and postharvest handling history could also have influenced the levels of occurrence of the investigated mineral elements. The reported variations in the occurrence levels of the investigated mineral elements has further emphasized the relevance for food composition analysis at the stage of marketing, if the supply of safe and quality foods to consumers is to be guaranteed. Statistical analysis which involved the use of Two-way ANOVA with multiple comparison, indicated statistical difference at P<0.05, between the levels reported for each of the mineral elements in the studied *Vernonia amygdalina* leaves.

3.2 Results for Selected Vitamins Analysis

The results for the determinations of the levels of ascorbic acid, thiamine, riboflavin and pantothenic acid in *Vernonia amygdalina* leaves obtained from some markets in Benin City are presented in Table 2 below. It is deducible from findings that the investigated vitamins were present in the studied samples though in varying amounts. Remarkably, the ranges of occurrence of the examined vitamins are: ascorbic acid (14.948±0.188 - 24.849±0.739) ppm; thiamine (2.278±0.174- 3.284±0.069) ppm; riboflavin (3.285±0.491 - 4.685±0.277) ppm and pantothenic acid (2.481±0.152 - 2.481±0.152) ppm. Thus from results, the highest occurring of the examined vitamins is ascorbic acid. On the other hand, the least occurring is pantothenic acid.

Table 2: Levels of Ascorbic Acid, Thiamine, Riboflavin and Pantothenic Acid in *Vernonia amygdalina* Leaves Obtained from Some Markets in Benin City

S/N	Sample source (markets)	Vitamins			
		Ascorbic acid ppm	Thiamine ppm	Riboflavin Ppm	Pantothenic acid ppm
1	New Benin	23.950±1.638	3.085±0.224	4.612±0.483	2.172±0.206
2	Ogida	18.749±2.541	2.836±0,743	3.972±0.953	2.079±0.874
3	Oliha	21.587±3.955	3.011±0.271	4.244±0.853	2.139±0.642
4	Ewosha	16.185±1.086	2.458±0.558	3.412±0.966	2.011±0.816

Table 2 (cont): Levels of Ascorbic Acid, Thiamine, Riboflavin and Pantothenic Acid in *Vernonia amygdalina* Leaves Obtained from Some Markets in Benin City

5	Okah	24.849±0.739	3.169±0.943	4.685±0.277	2.371±0.583
6	Useh	20.158±0.524	2.985±0.175	4.159±0.651	2.098±0.427
7	Uselu	26.845±0.846	3.284±0.069	4.724±0.158	2.481±0.152
8	Ekae	14.948±0.188	2.278±0.174	3.285±0.491	1.948±0.079
9	Oluku	19.528±3,471	2.903±0.288	3.918±0.629	2.118±0.395
10	Jeromi	22.479±0.492	3.108±0.671	4.258±0.469	2.169±0.815
11	Agbado	20.441±2.528	2.861±0.118	4.163±0.095	2.117±0.369
12	Egor	17.886±1.385	2.804±0.211	3.859±0.173	2.045±0.163

The noted variations in the levels of occurrence within individual vitamins are ascribed to differences in postharvest handling history especially marketing conditions, species, stage of maturity at the time of harvest and methods of their cultivation. According to Ogunmodede and Ukhun (1976) stage of maturity can also affect ascorbic acid level. It should be emphasized that in Nigeria, as it could be the case in some other developing countries of the world, foods marketing conditions are unstandardized. Hence routine post-harvest, particularly at the point of marketing investigations of foods for relevant nutrients including the vitamins examined in this work are imperative. This way, dangerously low levels of nutrients can be detected and a useful data base will then be available for institutional and home dietary formulations (Ukhun and Dibie, 1991).

It is nutritionally desirable that the investigated vitamins occurred in *Vernonia amygdalina* leaves. Apparently, it is an indication that *Vernonia amygdalina* leaves could be additional source of these vitamins to its consumers, especially as some individuals may not routinely have the traditional sources of these vitamins at their disposal. It is imperative to mention that *Vernonia amygdalina* leaves are an important component of the diets of some persons living in Nigeria, particularly the dwellers in the eastern region of Nigeria. Therefore, notwithstanding the low levels of these vitamins in the studied samples, it is important that their levels be considered in the determination of their intake in the population. Cumulative significance due to routine consumption of *Vernonia amygdalina* leaves is worthy of note. According to Ukhun and Dibie (1991) overall sources (i. e. cumulative intakes) of nutrients from different origins may be more important than single and isolated sources in the feeding habits of many people.

3.3 Results for Processing Studies

The results for the determinations of ascorbic acid, thiamine, riboflavin and pantothenic acid levels in sun dried and ground *Vernonia amygdalina* leaves are presented in Table 3 below.

Table 3: Results for Determinations of the levels of Ascorbic Acid, Thiamine, Riboflavin and Pantothenic Acid in Sun-dried and Ground *Vernonia amygdalina* Leaves

S/N	Parameter	Raw (fresh) sample	Sun dried ad ground sample
1	Ascorbic acids (ppm)	23.950 ±1.638	17.983±1.610
2	Thiamine (ppm)	3.085 ±0.224	2.753±0.118
3	Riboflavin (ppm)	4.612±0.483	0.958 ±0.172
4	Pantothenic acid (ppm)	2.172±0.206	0.605±0.141

It is discernible from Table 3 that there was a general reduction in the retention of the examined vitamins, following sun drying and grinding of the samples. The lowering of the levels of the investigated vitamins following sun drying and grinding, is an indication of negative relationship between the studied processing conditions and the examined vitamins. Clearly, instability of the vitamins to atmospheric conditions particularly to oxidative factors, should lead to their losses.

3.4 Results for Open Laboratory Storage Studies

The results for open laboratory storage studies with stored sun dried and ground *Vernonia amygdalina* leaves are presented in Table 4

Table 4: Results for Quantitative Analysis of Ascorbic acid, Thiamine, Riboflavin and Pantothenic Acid Constituents of *Vernonia amygdalina* leaves Stored under Laboratory Conditions for Two Months

S/N	Parameter	Sun dried and ground pre-stored sample	Sun dried and ground stored samples			
			Storage conditions/time (months)			
			Open Laboratory			
			Covered container		Opened container	
			1-month	2-months	1-month	2-months
1	Ascorbic acid (ppm)	17.983± 1.610	15.024 ± 1.305	13.120± 1.451	13.478± 1.152	10.646± 0.986
2	Thiamine (ppm)	2.753± 0.118	1.308± 0.226	0.752± 0.058	1.138± 0.095	0.397 ± 0.012
3	Riboflavin (ppm))	0.958 ± 0.172	0.391 ± 0.145	0.165 ± 0.082	0.283 ± 0.006	0.089 ± 0.014
4	Pantothenic acid (ppm)	0.605± 0.141	0.415± 0.106	0.268± 0.013	0.334± 0.071	0.1806± 0.011

It is deducible from Table 4 that progressive losses occurred in the levels of the examined vitamins in all the samples. The reported losses as findings indicated occurred more in samples stored in the opened container, which indicates that the covered container is a better storage facility than the opened container. This information will be relevant to handlers and users of Vernonia amygdalina leaves.

3.5 Results for Water Activity Studies

The results for water activity studies with ascorbic acid, thiamine, riboflavin and pantothenic acid constituents of sun dried and ground *Vernonia amygdalina* leaves are presented in Table 5

Table 5: Results for Water Activity (aw) Studies with Ascorbic acid, Thiamine, Riboflavin and Pantothenic Acid Constituents of Sun- dried, Ground and Stored *Vernonia amygdalina* leaves

S/N	Parameter	Sun dried and ground pre-stored sample	Sun dried and ground stored samples					
			Storage conditions/time (months)					
			$a_w 0.97$		$a_w 0.52$		$a_w 0.23$	
			1-month	2-months	1-month	2-months	1-month	2-months
1	Ascorbic acid (ppm)	17.983± 1.610	14.649± 1.283	11.572± 1.075	15.626± 1.106	14.188± 1.257	16.415± 1.363	15.226± 1.184
2	Thiamine (ppm)	2.753± 0.118	1198± 0.056	0.436± 0.125	1.644± 0.312	0.957± 0.144	1.982± 0.371	1.322± 0.161
3	Riboflavin (ppm)	0.958 ± 0.172	0.314 ± 0.035	0.097 ± 0.018	0.573 ± 0.098	0.215± 0.044	0.746± 0.135	0.429± 0.068
4	Pantothenic acid (ppm)	0.605± 0.141	0.352± 0.081	0.194± 0.017	0.477± 0.083	0.351± 0.069	0.528± 0.170	0.409± 0.142

Table 5 revealed that progressive reduction in the retention of the examined vitamins occurred in all the samples stored at the investigated water activity (a_w). It was particularly observed that storage losses of the examined vitamins occurred with increasing storage a_w. Remarkably, greater storage losses of the examined vitamins were recorded in the samples stored at the higher a_w than those of the samples stored at the lower a_w. The greater lowering effects of elevated a_w on the studied vitamins even if partly, is attributed to the enhanced amount of available water at the higher investigated a_w in this work. Clearly, greater available water would result in greater solubility of the water soluble vitamins the consequence of which it would appear is enhanced rates of their destruction. Furthermore, higher available water could have favoured the dissolution of oxygen in the studied samples, which subsequently, promoted the losses of the examined vitamins via oxidative processes. At the end of the study period, findings indicated that the examined vitamins were more stable in samples stored at a_w 0.23. This has further emphasized the central role of water activity in food stability. Significantly, water activity as reported by Rockland and Nishi (1980) is generally accepted to be more closely related to the physical, chemical and biological properties of foods and other natural products than is total moisture content.

4. CONCLUSION

This study investigated *Vernonia amygdalina* leaves for Cu, Co, Zn, Fe, Na, K, Ca, Mg, Cr, Se, ascorbic acid, thiamine, riboflavin and pantothenic acid. Findings indicated these food nutrients were present in the studied samples. Varying levels of the studied parameters were obtained in samples collected from different locations. It was also observed that the investigated processing and storage conditions led to reduced retention of ascorbic acid, thiamine, riboflavin and pantothenic acid. Further observed was that the investigated vitamins were more stable in samples store at a_w. The central role of a_w in food stability is thus emphasized.

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