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Studies with Selected Phytochemical Constituents of *Vernonia Amygdalina* Leaves and Influence of Processing and Storage

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Abstract

This work entailed in part, qualitative determinations of flavonoids, eugenols, steroids, and terpenoids constituents of processed and unprocessed *Vernonia amygdalina* leaves, quantitative investigations of the responses of flavonoids, terpenoids, steroids and anthocyanins constituents of *Vernonia amygdalina* leaves to sun drying and grating as processing methods, as well as the study of the influence of storage on the phytochemicals examined. Storage facilities were the open laboratory and at selected water activities (a_w), all at ambient conditions. *Vernonia amygdalina* Leaves samples used in this study were obtained from some markets in Benin City. Storage duration was two months. The various investigations carried out in this work were done using standard methods. Qualitative tests results indicated the presence of the phytochemicals examined in the various solvents extracts. Variations were however observed in their levels of occurrence. Quantitative results obtained for the phytochemicals examined in raw *Vernonia amygdalina* leaves were: flavonoids (1.62±0.20mg/g), steroids (1.15±0.37mg/g), anthocyanins (0.57±0.12mg/g), and terpenoids (0.02±0.01mg/g). Processing and storage treatments led to increase in the values of the phytochemicals examined. The noted increase were statistically significant ($P<0.05$) with respect to storage conditions. It is hoped that handlers and users of *Vernonia amygdalina* leaves would find this work relevant.

1.0 Introduction

Vernonia amygdalina (bitter) leaves are commonly used as additives in the preparation of many diets. Another remarkable use of *Vernonia amygdalina* leaves is the consumption of its aqueous extracts by some persons, apparently because of its varying medicinal values. Furthermore, some cultured fish farmers in Nigeria, use aqueous extracts of raw *Vernonia amygdalina* leaves as antimicrobial drug for treatment and control of microbial infections in their ponds. According to [1] *Vernonia amygdalina* leaves are rich sources of many nonnutritive bioactive secondary metabolites. It would appear however, that the gap in knowledge with respect to the rich heritage of *Vernonia amygdalina* leaves as material of high nutritional and therapeutic relevance, especially in its processed form, is hindering the maximum utilization of *Vernonia amygdalina* leaves. There is particular gap in knowledge with respect to processing and storage chemistry of *Vernonia amygdalina* leaves. The concern of this study is to fill this gap even if partly.

In Nigeria presently, processed and some stored products derived from *Vernonia amygdalina* leaves are sold in the open markets to consumers, even when unstandardized methods are used in the production and storage of these products. Processing subjects food to controlled conditions with the objective of altering it to a state of safety, durability and convenience; this is done by affecting targeted biological, physical and chemical parameters [2]. However, food processing can also create undesirable effects including loss of valuable nutrients and promotion of certain reactions with adverse consequences. The extent these undesirable effects will occur depends on both processing conditions and the compositional chemistry of the processed material. Depending on storage handling, undesirable reactions could also continue in the processed and stored products. Therefore, in considering any material whether for its nutritional or therapeutic significance, holistic approach inclusive of postharvest, processing and storage considerations is important.

The concern of this work will be in part, to carry out qualitative determinations of flavonoids, eugenols, steroids, and terpenoids constituents of processed and unprocessed *Vernonia amygdalina* leaves, then to investigate the responses of flavonoids, terpenoids, steroids and anthocyanins constituents of *Vernonia amygdalina* leaves to sun drying and grating as processing methods, as well as monitor the influence of storage in the open laboratory and at the studied water activities (a_w) on these phytochemicals. The storage quality of food does not depend on the water content, but on water activity (^{a}w) [3)]. Furthermore, the findings of [4,5,6,7,8,9,10,11,12,13,14,15,16,17,18] have shown that food stability, safety and other properties can better be predicted from a_w than from water content. Water activity in fact, plays central role in food stability.

Storage will be carried out under ambient conditions. Storage water activities will be at a_w 0.23, 0.52, and 0.97. Some other samples would be stored separately in opened and closed storage containers and kept in the open laboratory. All determinations would be carried out in accordance with standard methods. It is imperative to mention that literature reports on the effects of sun drying and grating, on the parameters of interest in the samples to be studied in this work, are scares if in existence. In particular, there is dearth need of information on the effect of a_w on the chemistry of sun dried, grated and stored *Vernonia amygdalina* leaves. Thus, findings from this study will fill existing gap in knowledge even if partly.

2.0. Materials and Methods

2.1 Sample Collection

Processed and unprocessed samples of *Vernonia amygdalina* leaves were purchased from five open markets viz:- Uselu, New Benin, Ogida, Santana, and Ikpoba hill (Orogbeni) markets, all in Benin City, Edo State. In each of the markets, five samples (purchased from five different sellers) both processed and unprocessed forms were obtained. The processed form of samples purchased, were the mashed and in wet form.

2.2 Samples Inspection and Cleaning

The unprocessed *Vernonia amygdalina* leaves used in this study were pretreated to free them from various forms of contaminants. Significantly, they were those free from disease (i.e. they were not affected by viral, bacteria or fungal infection). Additionally, contaminating plants and/or plants parts were identified and removed. Instructively, in all cases only healthy *Vernonia amygdalina* leaves were used in the study.

2.3 Samples Preparation

The various methods used to process and store samples in this work entailed initial sun drying of fresh *Vernonia amygdalina* leaves to constant weight. This gave the sun dried samples. Subsequently, the sun dried samples were grated, with the aid of Black and Decker 650W, BX550 blender. Thereafter, the grated 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 grated *Vernonia amygdalina* leaves were weighed in triplicates into separate 500ml glass beakers (Pyrex glass), and kept in air tight desiccators wherein a_w of 0.23, 0.52 and 0.97 respectively were established, in accordance with the method prescribed by [19]. Samples were stored for 2 months, and on monthly basis, they were investigated for the parameters examined in this work. All the storage desiccators were kept on laboratory bench at ambient conditions.

2.5 Measurement

2.5.1 Qualitative Phytochemical Screening of *Vernonia amygdalina* leaves Samples

2.5.1.1 Reagents

All the chemicals used in this study were BDH chemicals England; and they were of analytical grade.

2.5.1.2 Qualitative Screening Methods

Qualitative screening of the respective aqueous, methanol, ethanol, n-hexane, acetone and ethyl acetate extracts of the various samples for flavonoids, eugenols, steroids, and terpenoids were carried out in accordance with standard methods as described by some authors [20, 21, 22, 23, 24, 25, 26, 27].

2.5.1.3 Extraction

The methods of [28] were used to obtain the various crude *Vernonia amygdalina* leaves extracts. Extraction was carried, after samples of *Vernonia amygdalina* leaves were previously air – dried at room temperature for three weeks and subjected to particle size reduction with the aid of Black and Decker 650W blender. The extraction methods entailed weighing separately, 100g each of the test powdered samples into separate 500ml conical flasks containing 250ml of distilled water (aqueous extraction), 250ml of 99.5% methanol (methanolic extraction), 250ml of absolute ethanol (ethanolic extraction), 250ml of 85% n-hexane (hexane extraction), 250ml of 90% acetone (acetone extraction) and 250ml of 95% ethyl acetate (ethyl acetate extraction). The mixtures were covered and stirred every 24h using a sterile glass rod; storage time was 3 days in the case of aqueous extraction, and 5 days for each of methanolic, ethanolic, hexane, acetone and ethyl acetate extractions respectively. Subsequently, the respective mixtures were separately filtered through Whatman filter paper No. 1 (Whatman limited, England). The respective filtrates obtained were concentrated at 40 ^{0}C and subsequently, used for the various qualitative phytochemicals screening.

2.5.1.4 Test for Terpenoids

The qualitative test for terpenoids was carried out using Sakowski test as described by [22]. The procedure entailed addition of 2ml of chloroform to already measured 5ml of test extract in a test-tube, followed by the addition of 3ml of concentrated H_2SO_4. The presence of a reddish brown colouration at the interphase formed, indicated the presence of Terpenoids.

2.5.1.5 Test for Flavonoids
Qualitative determination of flavonoids was carried out in accordance with the method described by [27]. The test method entailed the addition of 1ml of 10% lead acetate to 1ml of test extract in a test – tube. Thereafter, the mixture was shaken gently and allowed to settle. Flavonoids presence was indicated by the formation of muddy brownish precipitate.

2.5.1.6 Test for Steroids.
The test for steroids was carried out by the addition of 10ml of chloroform to 1ml of the test extract, which dissolved the test extract. Thereafter, 10ml of concentrated Sulphuric acid was gently added along the walls of the test tube containing the solution of the test extract in chloroform; which subsequently formed a lower layer. The presence of steroids rings was indicated by a reddish brown colour at the interphase of the two layers [23].

2.5.1.10 Test for Eugenols
The test for eugenols was carried out by the addition of 5mls of 5% KOH to a measured 2ml of the test extract in a test – tube. Subsequently, the aqueous layer separated and was filtered. This was followed by the addition of three drops of 2M HCl to the filtrate. A pale yellow precipitate indicated positive test for the present of eugenols [24].

2.5.2 Quantification of Phytochemicals Examined
2.5.2.1 Extraction of phytochemicals: The phytochemicals examined were extracted with methanol with the aid of soxhlet extractor. The methanol used was purchased from Sigma –Aldrich (Steinheim, Germany).
The details of the extraction process, is described below: One hundred grams of ground plant material was weighed and then uniformly distributed in a thimble. Subsequently, 400ml of methanol (material: solvent ratio is 1:4) was measured into the extraction flask and the extraction was thereafter carried out for 20h (it was observed that the solvent in the siphon tube of the extractor became colourless after 18h of extraction, but the extraction was continued for another 2h to ensure that exhaustive extraction has been carried out). Subsequently, the methanol was removed with the aid of rotary evaporator (RE52-3 SEARCH TECH INSTRUMENTS).

2.5.2.2 Determination of Total Flavonoids Content
Total flavonoid content was determined colorimetrically in accordance with the method described by [30, 31].

2.5.2.2.1 Reagents
Methanol, aluminium chloride, potassium acetate and quercetin were obtained from Sigma – Aldrich (Steinheim, Germany). Distilled water was obtained using a Milli – Q system (Millipore – Bedford, MA, USA). Except otherwise stated, all chemicals used were analytical grade.

2.5.2.2.2 Procedure
To 0.5ml methanolic extract of sample and standards (quercetin solutions of concentration : 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10mg/l; prepared by dissolving quercetin in methanol) in separate test tubes, were added 4.5ml of methanol, and shaken thoroughly, after which 0.1ml of 10% aluminium chloride was added to each of the test tubes. This was followed by the addition of 0.1ml of 1M potassium acetate,

after which the contents of the various test-tubes were thoroughly mixed. Thereafter, the test – tubes and their respective contents were allowed to stand for 30min at room temperature. Blank was also prepared. Subsequently, the absorbance of the reaction mixture was read at 415nm, using Uv/vis spectrophotometer (Jenway spectrophotometer, 6715 Uv/vis). The total flavonoid content was calculated from the standard graph of quercetin, and the results were expressed as quercetin equivalent (mg/g). Quercetin is a common reference compound for total flavonoids determination.

2.5.2.3 Determination of Total Terpenoid Content

Total terpenoids was spectrophotometrically determined in accordance with the method of [32].

2.5.2.5.1 Reagents

Chloroform, Sulphuric acid, and Linalool were purchased from Merck (Germany). Methanol was obtained from Sigma – Aldrich (Steinheim, Germany). Distilled water was obtained using a Milli – Q system (Millipore – Bedford, MA, U. S. A). Except otherwise stated, all chemicals used were analytical grade.

2.5.2.5.2 Procedure

To 1ml of the methanolic extract of the test plant, in different test–tubes was added 3ml of chloroform. Subsequently, the content of each of the test tubes was thoroughly shaken and allowed to stand for 3mins. Thereafter, 0.2ml of concentrated sulphuric acid was added to each of the test – tubes, after which they were incubated in the dark, at room temperature for 2h (a reddish brown precipitated was formed during incubation). At the end of the incubation period, the supernatant of the reaction mixture in the various tests – tubes were separated by gentle decantation approach. Subsequently, 3ml of 95% (v/v) methanol was added to the precipitate in each of the test tubes, they were thereafter, shaken until the respective precipitates were noted to have completely dissolved in methanol. The standard solutions (Linalool dissolved in methanol and of concentrations: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10mg/l), the various samples and blank absorbance readings were carried at 538nm, using Uv/visible spectrophotometer (Jenway spectrophotometer, 6715 Uv/vis). The total terpenoids content was calculated from the standard graph of linalool, and the results were expressed as linalool equivalent (mg/g).

2.5.2.3 Determination of Steroids Content

Quantitative determination of steriods in the sample was spectrophotometrically carried out in accordance with the method described by [33].

2.5.2.3.1 Reagents

Cycloartenol, sulphuric acid, Iron (III) chloride and potassium hexacyanoferrate (III) were purchased from Sigma-Aldrich (Steinheim, Germany). Water was obtained using Milli-Q system (Millipore Bedford, MA, USA).

2.5.8.2 Procedure

To 1ml each of standard cycloartenol solutions, blank and methanolic extract of investigated plant, in separate 10ml volumetric flasks, was added 2ml of 4M sulphuric acid and then 2ml of 0.5% (w/v) of Iron (III) chloride. This was followed by the addition of 0.5ml of 0.5% (w/v) of potassium hexacyanoferrate (III) solution. After each addition, the contents of the various flasks were thoroughly shaken. The respective volumetric flasks with the mixture contained therein were subsequently heated for 30min in a thermostatic water bath maintained at 70 ^{o}C. During the heating stage, the flasks were shaken at intervals of 5min for 30 sec. At the end of the heating period, the flasks and their respective

contents were cooled to room temperature. Thereafter, the content of each of the volumetric flasks was made up to the 10ml mark with distilled water. Subsequently, absorbance reading was taken at 780nm using an Ultraviolet-visible spectrophotometer (Jenway spectrophotometer 6715 Uv/vs). The respective steroid contents of each of the extracts were calculated, using value obtained by extrapolation from the standard graph of cycloartenol.

2.5.2.4 Determination of Total Anthocyanin Content

Total anthocyanin content was carried out using the method described by Lima *et al* (2012) as reported by [34].

2.5.2.4.1 Reagents

Methanol, Trifluoroacetic acid and Cyanidin 3-0-glucoside were purchased from Sigma-Aldrich (Steinheim, Germany). Water was obtained using Milli-Q system (Millipore-Bedford, MA, USA).

2.5.2.4.2 Procedure

One millilitre of methanolic extract of investigated plant was measured into a 10ml glass tube. Thereafter, the volume was made up to 3ml, by the addition of methanol acidified with 0.1% (v/v) trifluoroacetic acid. The tube was shaken and subsequently, absorbance reading was taken at 530nm, using an Ultraviolet/visible spectrophotometer (Jenway Spectrophotometer 6715 Uv/vis), against a blank containing a mixture of methanol and 0.1% (v/v) trifluoroacetic acid. Extrapolation of the concentration of authocyanin in the sample was carried out from a standard curve of cyanidin 3-0-glucoside, and the value contained was expressed as mg cyanidin 3-0-glucoside/g of sample.

3.0 Results and Discussion

3.1: Qualitative Phytochemical Determinations

Results for the qualitative tests for the secondary metabolites examined including flavonoids, eugenols, steroids, and terpenoids levels in the samples of *Vernonia amygdalina* leaves obtained from selected markets in Benin City and using aqueous, methanol, ethanol, n-hexane, acetone and ethyl acetate as extracting solvents are presented in Table 1.

As shown in Table1, qualitative test for flavonoids in the aqueous, methanol, ethanol, n-hexane, acetone and ethyl acetate extracts of *Vernonia amygdalina* leaves indicate its occurrence in abundance. However, in samples 3 and 4, slight occurrence of flavonoids was observed in their respective aqueous, methanol, ethanol, n-hexane, acetone and ethyl acetate extracts. It is imperative to mention that the various samples were obtained from different locations which could be a contributory factor to the results obtained; furthermore, the observed variations in flavonoids levels could additionally, be due to different biological activities including their rates of production and utilization, in the various *Vernonia amygdalina* leaves studied.

Also discernable from Table 1, are that eugenol and steroids in the aqueous, methanol, ethanol, n-hexane, acetone and ethyl acetate extracts of the *Vernonia amygdalina* leaves, had similar pattern of occurrence. Staggering pattern of occurrence of terpenoids, which appeared to be related to samples of *Vernonia amygdalina* leaves was observed. Significantly, in samples 1, 2 and 4, extracts of *Vernonia amygdalina* leaves obtained, using aqueous, methanol, ethanol, n-hexane, acetone and ethyl acetate separately, indicate abundant presence of terpenoids. On the other hand, in similar solvents extracts of samples 3 and 5, terpenoids occurred slightly. Apparently, this appears to be an indication that

variations could exist in the levels of certain secondary metabolites found in *Vernonia amygdalina* leaves marketed in Benin City. Soil type, agricultural method of cultivation, maturity at the time of harvest, as well as postharvest handling methods, could even if partly, be responsible for this.
The observed variations in pattern of occurrence of some of the secondary metabolites investigated in the various *Vernonia amygdalina* leaves, is worthy of note. This is particularly important, as the variation tended to be sample related. Suffice it to say that, consistency of industrial applications and dietary preparations could be compromised, if the right species of *Vernonia amygdalina* leaves are not identified and used. In Nigeria, *Vernonia amygdalina* is one of the crops cultivated domestically and in the farmland. Therefore, greater variations could exist in terms of species, soil types and locations; as well as agricultural practices, including fertilizer applications especially as it relates to type, amount, and time of application. The maturity of the plant before harvest could also be relevant.

3.2: Quantitative Phytochemical Determinations

Results of quantitative analysis of the non-nutritive bioactive substances examined in raw, sun dried, grated and stored *Vernonia amygdalina* leaves are presented in Table 2. Quantification of Selected non-nutritive Bio-active Substances (Phytochemicals) Contents of Raw, Sun Dried, Grated and Stored *Vernonia amygdalina (*Bitter*)* Leaves.

Table 1: Results for the qualitative tests for the examined secondary metabolites including flavonoids, eugenols, steroids, and terpenoids levels in *Vernonia amygdalina* leaves samples obtained from selected markets in Benin City

		Extracts					
S/N	**Phytochemicals /samples**	**Aqueous**	**Methanol**	**Ethanol**	**n-Hexane**	**Acetone**	**Ethyl acetate**
1	Flavonoids						
	*sample 1	++	++	++	++	++	++
	*sample 2	++	++	++	++	++	++
	*sample 3	+	+	+	+	+	+
	*sample 4	+	+	+	+	+	+
	*sample 5	++	++	++	++	++	++
2	Eugenols						
	*sample 1	+	+	+	+	+	+
	*sample 2	+	+	+	+	+	+
	*sample 3	+	+	+	+	+	+
	*sample 4	+	+	+	+	+	+
	*sample 5	+	+	+	+	+	+
3	Steroids						
	*sample 1	+	+	+	+	+	+
	*sample 2	+	+	+	+	+	+
	*sample 3	+	+	+	+	+	+
	*sample 4	+	+	+	+	+	+
	*sample 5	+	+	+	+	+	+
3	Terpenoids						
	*sample 1	++	++	++	++	++	++
	*sample 2	++	++	++	++	++	++
	*sample 3	+	+	+	+	+	+
	*sample 4	++	++	++	++	++	++
	*sample 5	+	+	+	+	+	+

+ = Slightly present, ++ = Largely present, - = Absent

Table 2: Results for the quantitative tests for the examined secondary metabolites including flavonoids, eugenols, steroids, and terpenoids levels in *Vernonia amygdalina* leaves samples obtained from selected markets in Benin City

S/N	Parameter	Raw (fresh) sample	Sun dried and pre-stored sample	Stored samples									
				Storage conditions/time (months)									
				a_w0.97		a_w0.52		a_w0.23		Open Laboratory			
										Covered container		Opened container	
				2-months	1-month	2-months	1-month	2-months	1-month	2-months	1-month	2-months	1-month
1	Flavonoids (mg/g)	1.62 ± 0.20	1.91 ± 0.44	4.16 ± 1.10	2.38 ± 0.71	5.54 ± 1.06	3.15 ± 0.71	7.78 ± 1.32	4.68 ± 0.30	4.79 ± 0.81	2.73 ± 0.61	2.13 ± 0.15	2.05 ± 0.46
2	Steroids (mg/g)	1.15 ± 0.37	1.16 ± 0.20	3.01 ± 0.65	2.06 ± 0.41	4.07 ± 0.92	2.65 ± 0.14	5.72 ± 1.08	3.40 ± 0.60	3.59 ± 0.18	2.46 ± 0.30	1.48 ± 0.12	1.25 ± 0.44
3	Terpenoids (mg/g)	0.02 ± 0.01	0.02 ±0.01	0.06 ±0.03	0.03 ±0.01	0.09 ±0.03	0.05 ±0.01	0.12 ±0.02	0.08 ±0.04	0.07 ±0.03	0.04 ±0.02	0.03 ±0.01	0.02 ±0.01

Findings indicate that flavonoids, steroids, terpenoids, and anthocynanins were present in varying amounts in raw *Vernonia amygdalina* leaves. Quantitatively, amongst the phytochemicals examined in raw *Vernonia amygdalina* leaves, flavonoids with a value of (1.62±0.20mg/g) occurred highest. In decreasing order of occurrence with respect to the other phytochemicals examined is steroids (1.15±0.37mg/g) anthocyanins (0.57±0.12mg/g), and terpenoids (0.02±0.01mg/g). According to [35, 36] the factors which influence the composition of foods of plant origin include genetic constitution, method of propagation, growing conditions, age or maturity at the time of harvest, as well as length and condition of storage before use. It would appear that these factors influenced the respective occurrence of the phytochemicals examined in samples of *Vernonia amygdalina* leaves used in the study.

Also discernable from results presented in Table 2, was that sun drying of *Vernonia amygdalina* leaves led to varying increases in all the phytochemicals examined; except terpenoids whose value of 0.02±0.01mg/g in the raw sample, did not change following sun drying of *Vernonia amygdalina* leaves. Further observed from results (Table 2) was that storage of sun dried and grated *Vernonia amygdalina* leaves in the open laboratory under ambient conditions, farvoured further biosynthesis of flavonoids, steroids, terpenoids and anthocyanins in both closed and opened samples storage containers. Evidently (Table 2), these phytochemicals increased in amount at the end of the two months storage duration. Additionally, results (Table 2) indicated that at the end of the two months of storage, samples kept in the closed samples storage containers had higher concentrations of the phytochemicals examined, compared to those of the samples kept in the opened samples storage containers. The implication here is that the conditions in the closed samples storage containers favoured more, the further biosynthesis of the phytochemicals examined in sun dried, grated and stored *Vernonia amygdalina* leaves, than when samples are kept in opened samples storage container and stored in the open laboratory.

Water activity studies with sun dried, grated and stored *Vernonia amygdalina* leaves, has revealed that in storage, there was continued biosynthesis of flavonoids, steroids, terpenoids and anthocyanins, at the water activities (a_w) investigated. Further deducible from the results presented in Table 2 was that the phytochemicals examined in the samples stored at a_w 0.23 recorded the highest level of increase. The pattern of the results was such that with increasing a_w, the level of increases in the phytochemicals examined decreased. In particular, increases in amount were lowest in the phytochemicals examined in samples stored at the very high a_w of 0.97. It would appear therefore, that the prevailing conditions at the low a_w of 0.23, favoured more of the series of reactions that led to increases in the phytochemicals examined, vis-à-vis the degradation reactions; far and above the conditions at the higher a_w of 0.52 and 0.97. The noted storage increases at the different storage conditions were found to be statistically significant ($P<0.05$).

As results in this study revealed, sun drying, as well as the various storage conditions investigated, farvoured the mechanisms involved in the production of the phytochemicals examined in *Vernonia amygdalina* leaves. It is further opined that the possible non/or reduced utilisations of the phytochemicals examined, in other biological activities in the stored samples, also led to their respective accumulations.

It is remarked by [37] that in milled cowpea flour, physical attributes such as large surface area, high degree of porosity, enzyme decompartimentalization following milling and the milling operation which is a form of stress, could have promoted chemical responses. With respect to this study, it is considered that the grating of the sun dried *Vernonia amygdalina* leaves studied, prior to storing them, accentuated wide array of chemical reactions, particularly as histological disintegration, as well as enhanced enzyme decompartmentalization usually accompany grating operations. It would appear therefore, that these events promoted biosynthesis of the phytochemicals examined in the processed.

In many Nigerian folk medicines fresh plants materials are administered to patients. In some other situations, patients are advised to store certain plants parts in either aqueous or alcoholic medium.

The believed is that the efficacy and certain organoleptic properties of these herbal compositions change with storage. The common explanation to such changes in the efficacy and organoleptic properties of the prepared herbal product is that more extractions took place in the stored materials. Clearly, in addition to possible extraction, this work has shown that further biosynthesis of the phytochemicals examined occur postharvest and in storage.

4.0. Conclusion

Findings from this work have revealed that the phytochemicals examined are present in *Vernonia amygdalina* leaves. Furthermore, in *Vernonia amygdalina* leaves, increases in the phytochemicals examined were noted when sun dried and grated *Vernonia amygdalina* leaves were stored. Therefore, users of sun dried and stored *Vernonia amygdalina* leaves should be conscious of the continued biosynthesis of the phytochemicals examined in this study, so as to ensure that their concentrations remain consistent in their various applications.

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