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Review Article
Pre-harvest and Post-harvest Factors Affecting Bioactive Compounds From *Vernonia amygdalina* (Del.)

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Abstract

Vernonia amygdalina (VA), a dark green leafy vegetable, is a rich source of chemicals and bioactive compounds such as phenols, flavonoids, terpenoids, resins, essential oils and some vitamins. These compounds are reported to have diverse health benefits including antimicrobial and metabolism regulatory properties. Most of the compounds are found mainly in the leaves of VA and the juice extracted therefrom. The leaves are commonly consumed as fresh vegetable or the fresh juice drank as tonic/medicine. In addition, the extracts are used in breweries as a substitute for hops. The concentration and stability of these bioactive compounds are nonetheless affected by pre-harvest factors such as phase of development, cultivar, fertilizer application, agro-climatic conditions, on the one hand and postharvest factors such as processing, solvent extraction treatments and storage, on the other hand. This review discusses the pre-harvest and post-harvest factors that influence the functional properties of VA.

Key words: *Vernonia amygdalina,* preharvest, postharvest, bioactive compounds, processing

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

INTRODUCTION

Vernonia amygdalina (Del.) (VA) known commonly as 'bitter leaf' is a perennial shrub belonging to the Asteraceae family[1]. The plant's bark is rough and marked by dense black straits. The leaves are elliptic, about 6 mm in length, dark green in color and (when fresh) have an odor and bitter taste that distinguishes it from other plants[2]. *Vernonia amygdalina* is widely grown in countries such as Nigeria, Benin, Togo, Cameroun, Ghana, Gabon, Democratic Republic of Congo, Zimbabwe, South Africa, Ethiopia, Rwanda, Tanzania, Uganda, Kenya, Swaziland, China and Malaysia and in some parts of South America[3-9]. The leaves are sold or exported to several countries of the world as fresh, frozen, or dried and powdered herbs[8]. The plant is known by different names in different countries. Some of such names are: Shikawa, ikaruga, chrysanthemum tonsils, mpasi nyioso, ndoki, onugbu, ewuro, umubilizi, omubirizi etc[9,10].

Vernonia amygdalina is one of the oldest known vegetables and contains a lot of compounds that are of immense medicinal value. Scientific studies have shown that various extracts from VA possess various pharmacological properties, including anthelmintic, antiprotozoal, antibacterial, antitumor and antiplasmodial activities[11-17]. The beneficial effects of the plant leaves in managing several chronic diseases[18,19], including blood glucose and lipid modulating properties of the leaves in experimental animals[20,21] and humans[1,2,22] have also been reported. Extracts of VA leaves have been shown to contain polyphenols which include terpenoids, saponins, flavonoids, steroids, tannins, anthraquinones, phenols, alkaloids etc (Table 1)[23-31]. *Vernonia anygdalina* when pre-treated, has a special aroma and bitter-sweet taste in soups which is always desired and cherished in many cultures. Its usage in food and medicine is well rooted in African history and is gaining importance outside Africa, in countries such as Malaysia and China. Indeed, there has been increased global awareness on the importance of VA as a medicinal plant and many researchers and food processors are investigating the potentials of its extracts and phytochemicals and optimizing their exploitation in health preservation and enhancement and in disease management[1].

Table 1: Health benefits of bioactive and chemical compounds found in *Vernonia amygdalina* leaves

Compounds	Concentration (dry weight)	Health and Food benefit	References
Vitamin C	44%	Antioxidant	Abioye *et al.*[23]
	166.5 mg/100 g		Ejoh *et al.*[24]
Vitamin A	30.0 mg/100 g	Immunity, vision	Ejoh *et al.*[24]
Phenol	50 mg/100 g	Antioxidant, anticancer, anti-inflammatory	Abioye *et al.*[23]
	0.97 (mg GAE/g of extract)	antifungal and antibacterial effects	Oriakhi *et al.*[25]
	160 mg L^{-1}		Imaga and Bamigbetan[26]
	0.25%		Ndukwe *et al.*[27]
Steroids	0.17%		Ndukwe *et al.*[27]
Saponins	63.33 mg/100g	Antioxidant, anticancer, anti-inflammation, antiparasitic	Abioye *et al.*[23]
	0.64%		Ndukwe *et al.*[27]
Flavonoid	65.4 (mg QE/g of extract)	Antioxidant, antimicrobial, anticancer, antiinflammation,	Oriakhi *et al.*[25]
	470 mg L^{-1}	antiparasitic, anti-allergic, anti-inflammatory and	Imaga and Bamigbetan[26],
	-	anti-microbial activities	Erasto *et al.*[28] and Igile *et al.*[29]
	0.47%		Amodu *et al.*[30]
			Ndukwe *et al.*[27]
Tannins	230 mg L^{-1}	Antioxidant	Imaga and Bamigbetan[26]
	-	Antitumoral effect	Amodu *et al.*[30]
	0.74%		Ndukwe *et al.*[27]
Alkaloids	-	Antitumoral, analgesic, antispasmodic and bactericidal effects	Amodu *et al.*[30];
	2.78%		Ndukwe *et al.*[27]
Anthraquinone	-	Antitumoral effect	Amodu *et al.*[30]
Protein	19.2 mg/100 g	-	Ejoh *et al.*[24]
Sugar	14.3 mg/100 g	-	Ejoh *et al.*[24]
Fat	4.7 mg/100 g		Ejoh *et al.*[24]
	7%		Adama *et al.*[31]
Iso-Alpha Acid	8.52 mg L^{-1}	Antiseptic and preservative properties	Adama *et al.*[31]
Alpha Acid	9.27 mg L^{-1}	Antiseptic and preservative properties	Adama *et al.*[31]
Resin	20.4%	Taste	Adama *et al.*[31]
Essential Oil	1.2%	Preservative properties	Adama *et al.*[31]

Several pre-treatment methods are adopted in the processing and extraction of various products from the leaves of VA. These include sun drying, squeezing, freezing, blanching, boiling, oven drying, soaking and fermentation. These processing methods influence the quality attributes of VA. There is a large body of literature on the availability and pharmaceutical potentials of many phytochemicals from VA[1,9,18-22,32-37] both for human and animal use. However, the type of post-harvest treatment adopted for VA, especially the leaf, affects its functional properties[1,18,19,31,38]. Understanding the role of pre-harvest and post-harvest treatment on VA will clearly guide researchers as they search for phytopharmaceuticals in the plant (for example, pointing to the direction of gaps in existing knowledge and guiding the choice of methodologies to be adopted). Such understanding and knowledge will also guide product development from the plant. Therefore, the aim of this review was to describe and discuss the pre-harvest and post-harvest factors influencing the chemical and bioactive compounds in *Vernonia amygdalina* (Del).

METHODS FOR DATA MINING AND SYNTHESIS

The online scientific literature was searched in February, 2016 (using the Google scholar database). The key words "*Vernonia amygdalina*" and "bitter leaf" were used for the search. From the initial search results, 379 full text articles were identified. These articles were further scrutinized and those that were directly relevant to this review (65 in number) were selected. The reference lists of the selected articles were cross-checked for omitted articles and 5 other relevant articles were identified. These were sought for and obtained, online (2 articles) or through e-mails to their authors (3 articles). Relevant articles already in the possession of the authors but not obtained from the online search, (6 articles) were also used. From all of these, 76 articles were included and the relevant information in them were synthesized and collated.

CULTIVATION AND POST-HARVEST TREATMENT OF *Vernonia amygdalina*

Vernonia amygdalina (Del.) can be cultivated, preserved and processed in many ways depending on the intended usage. It is widely grown in farms, plantations, along the fencing of homes, or drainage. It also grows wild in the forests[33]. *Vernonia amygdalina* is farmed in Nigeria, Cameroun and several other countries in Africa and Asia. However the more popular growth environment is around the homes because of the ease of harvesting. Processing and preservation of VA leaves depends on the end use. Propagation of VA is mainly by cutting, layering or *in vitro* micro-propagation[32]. Though VA is draught-resistant, it thrives best during the rainy period[34-39]. The leaves of VA are manually harvested usually with the hands and conveyed to the processing point either in baskets or sacks. *Vernonia amygdalina* leaves have two notable applications; in nutrition and in medicine. Locally, the bitter juice is extracted from the leaf (to reduce the bitterness) if it is to be used in cooking. The bitter extract is drunk as medicine and for its tonic effect. Several pre-treatment methods such as drying, boiling, blanching, fermentation, freezing, etc., can be applied to the leaves depending on the desired nutritional and/or medicinal property[37,38]. Many of the pre-treatment methods prior to further processing of VA are temperature dependent. However VA leaves are usually milled in the fresh form or dried before solvent extractions[39-44]. The predominant treatment method is sun-drying or air-drying at room temperature, followed by milling.

Effect of processing methods on the functional properties of *Vernonia amygdalina*: *Vernonia amygdalina* (Del.) is subjected to immediate physical and physiological deterioration when harvested. These changes can result in loss of water and discoloration. The extent of changes such as discoloration is a primary factor in determining the quality attributes of most medicinal plant by consumers[45]. These changes can be delayed to some extent by the application of several pre-treatment methods[46-48] before further processing. Application of postharvest treatments such as drying, freezing, salting and heat treatment on medicinal plants before juice extraction has been reported to affect the appearance, nutritional quality and bioactive compounds of some medicinal herbs and fruits[46-51].

Drying: Melariri *et al.*[41] reported that drying is the most fundamental postharvest preservation method for medicinal plants because of its simplicity and conservation of the quality attributes of the plants. *Vernonia anygdalina* is grown mostly in the tropics, consequently, sun-drying or indoor air-drying is the cheapest preservation method adopted because of the abundance of solar energy in that climatic zone. Temperature management is very important in the drying of medicinal plants. This often impacts on the energy cost of the drying process. The utilization of solar energy in drying is a very cheap alternative though it takes longer time due to the vagaries of weather. However Ejoh *et al.*[24]

Fig. 1(a-b): (a) Fresh and (b) Sun-dried leaves of *Vernonia amygdalina* (Del.)

have shown that drying has notable influence on the functional properties of VA such that if artificial drying is adopted, low temperatures are utilized.

Sun drying has been found to decrease the percentage gelation and foaming capacity of VA while it increases its solubility, water absorption capacity and swelling power. It nonetheless has no effect on the foaming stability of VA[38]. Drying (solar, sun and oven drying) alters the nutrient composition of VA[52]. Qualitative photochemical analysis of ethanol extract from the sun dried leaves of VA shows that the concentration of flavonoids and steroids reduced while tannins, alkaloids, saponins and phlabotannins increased in concentration[53]. The impact of sun-drying on VA is shown in Fig. 1. The degradation of chlorophyll is evident in the color change from deep green to very light green, reducing the acceptability of the leaf as vegetable.

Salting and fermentation: The nutrient composition, phytochemical composition and organoleptic characteristics in fresh VA leaves are affected by preservation by salting. Oboh *et al.*[54] reported a dull dark green discoloration of the fresh leaves when preserved with low concentration of table salt, brine or mixture of brine and vinegar. Higher concentrations of table salt, however, preserved the bright dark green color. Although Oboh *et al.*[54] did not mention the actual concentrations of the various salts used, they found that heavy salting preserved the initial physiological structure of the leaves. This implies that pH is important in the preservation of color and texture of fresh VA leaves. Substantial decreases of up to 20-98% in protein, carbohydrate, lipid, vitamin C and β-carotene in fermented VA leaves has been reported[54,55] and this might be due to the leaching of the nutrients into the aqueous medium. Ifesan *et al.*[56] also reported losses of vitamin C and thiamine in fermented VA leaves while their niacin and mineral contents increased. There were also losses of steroids in the preservation of the leaves with brine, vinegar and high concentration of table salt[56]. A decrease of 36-46% in total phenol content of the leaves by various salt applications was reported. Salting and fermentation also prevented or inhibited the growth of *Aspergillus niger*, *Penicillium* spp. and *Fusarium* spp. but had no effect on *Aspergillus flavus*[54]. Losses in vitamins as a result of fermentation and other processing methods have also been reported[55-60] (Table 2).

Effect of solvent extraction: Various studies have shown that the solvent used in phytochemical extraction affects the stability, presence or absence of bioactive compounds in VA[60-63]. For example, the type of solvent used in the extraction of phyto-compounds has been found to have great influence on the stability and presence of phlobatannins, tannins, alkaloids, terpenoids, cardiac glycosides and flavonoids[60,61]. Management of the extraction procedures is therefore very important in maintaining or enhancing the presence of bioactive compounds because most of the compound are heat-labile and are easily susceptible to degradation in various media. Saponins, unlike other phytochemicals have been found to be relatively stable in most of the extraction solutions[61,63]. Adebayo *et al.*[62] found various levels of degradation in bitter leaves harvested from Northern Ghana and extracted with water, ethanol, methanol and petroleum ether. They reported that methanol extracts yielded higher percentages of saponins, terpenoids, tannins, phenols, alkaloids and flavonoids and showed that terpenoids, tannins, phenols, alkaloids and flavonoids were degraded completely in aqueous solution while only tannins, terpenoids and phenols were degraded completely in petroleum ether. However Ghamba *et al.*[63] reported a lower

Table 2: Effect of processing methods on the functional properties of *Vernonia amygdalina*

Methods of processing	Plant part	Key finds	References
Drying			
Sun drying	Leaf	↓% gelation, forming capacity, anthraquinone, phosphorous content. No effect on foaming stability ↑solubility, water absorption capacity and swelling power, crude protein, lipid, calcium, potassium, sodium and nitrogen content. Flavonoids and steroids, tannins, alkaloids and phlabotannins.	Aletor *et al.*[42], Aliero and Abdullahi[52] and Wazis *et al.*[53]
Solar drying	Leaf	↑crude protein, lipid, calcium, potassium, sodium and nitrogen content. ↓phosphorous content	Aliero and Abdullahi[52]
Oven drying	Leaf	↑crude protein, lipid, calcium, potassium, sodium and nitrogen content. ↓phosphorous content	Aliero and Abdullahi[52]
Salting and fermentation			
Low salting	Leaf	Discoloration to dull dark green. Loss of steroid, phenol. Inhibition of growth of *Aspergillus niger, Penicillium* spp. and *Fusarium* spp., but no effect on *Aspergillus flavus*	Oboh *et al.*[54]
Heavy salting	Leaf	Preserved the bright green color. Loss of steroids, phenol. Inhibition of *Aspergillus niger, Penicillium* spp. and *Fusarium* spp., but no effect on *Aspergillus flavus*	Oboh *et al.*[54]
Salt and vinegar	Leaf	Discoloration to dull dark green color. Loss of steroids, phenol. Inhibition of *Aspergillus niger, Penicillium* spp. and *Fusarium* spp., but no effect on *Aspergillus flavus*	Oboh *et al.*[54]
Fermentation	Leaf	↓protein, carbohydrate, lipid, vitamin C, β-carotene and thiamine.	Oboh and Madojemu[55], Oboh *et al.*[54] and Ifesan *et al.*[56]
Blanching, boiling and mechanical abrasion	Leaf	Loss of vitamin C. ↓phenol and saponins content. Loss of phosphorus, potassium, calcium and iron; β- carotene, protein, minerals, vitamins A, B_1, B_3, C and E discoloration	Adetuyi *et al.*[57], Alabi *et al.*[58], Abioye *et al.*[23], Ejoh *et al.*[24] and Tsado *et al.*[59]

concentration of flavonoids from ethanol extracts compared to aqueous extracts from VA leaves, a finding that is contradictory to the report of Adebayo *et al.*[62].

Blanching, boiling and mechanical abrasion: *Vernonia amygdalina* (Del.) is subjected to different de-bittering processes to remove astringent components before it is used for cooking. This ranges from soaking, blanching and abrasion [including squeezing with and without salt (NaCl) and palm oil][57,64,65]. Abioye *et al.*[23] found that though unprocessed VA leaves can provide up to 90 mg day^{-1} of vitamin C, blanching and abrasion with salt or palm oil caused up to 58% percentage loss of vitamin C in the leaves. Drastic reduction in phenols and saponins contents of up to 64% especially in blanched VA leaves has been reported[31]. Blanching, boiling and mechanical abrasion elevate the temperature of the leaf which might account to the degradation of the vitamin C and the other heat labile phytochemicals. Furthermore, Ejoh *et al.*[24] and Tsado *et al.*[59] independently reported various degrees of losses of minerals such as phosphorus, calcium, potassium and iron in boiled, blanched, squeezed and dried VA leaves, with squeezing having the highest negative effect on the minerals. Loss of β-carotene, protein, minerals and vitamins A, B_1, B_3, C and E have also been reported in VA leaves squeezed with or without salt[59,65].

PACKAGING AND COLD STORAGE

Temperature management methods are important for the maintenance of quality attributes (including chemical components) of medicinal and aromatic plants. However, the available information on storage temperature of VA leaves is limited. Temperature and storage period have been found to have influence on soft resin, alpha and iso-alpha acid constituents of VA leaves[66]. They were more stable in storage at 5°C compared to 27°C when stored up to a period of 1-6 months[24]. Musa and Ogbadoyi[67] found various ranges of losses in beta carotene, vitamins, oxalate and mineral content of VA leaves stored at -4°C for 2-4 weeks.

AGRO-CLIMATIC EFFECT ON THE FUNCTIONAL AND BIOACTIVE PROPERTIES OF *Vernonia amygdalina*

Vernonia anygdalina is grown in several agro-climate zones, yet the specific effect of these different agro-climatic zones on the bioactive and functional properties of VA is scarcely known. However there are reports indicating that growth and cultivation conditions can affect the functional and bioactive properties of medicinal plants[47,68-72]. Imaga and Bamigbetan[26] reported an absence of phlobotanin in fresh VA leaves harvested from Lagos State, South West Nigeria, while other bioactive compounds such as tannins,

Table 3: Possible effects of agro climatic variations on the functional and bioactive properties of *Vernonia amygdalina*

Location of harvest	Detected compounds	References
Lagos, South-West Nigeria	No phlobatanin. tannins, flavonoids, alkaloids and cardiac glycosides, present	Imaga and Bamigbetan[26]
South-South Nigeria	Absence of flavonoids and alkaloids. Tannins, saponins and glycosides, present	Oboh *et al.*[54]
North Central Nigeria	Presence of alkaloids, flavonoids, tannins, cardiac glycosides, saponins, free anthraquinone and anthracyanosides	Amodu *et al.*[30]
South East Nigeria	Presence of phenol, flavonoids, tannins, saponins and steroids	Ndukwe *et al.*[27]
North East Nigeria	Absence of phenol and volatile oil. Presence of saponins, alkaloids, flavonoids, anthraquinone, tannins, terpenoids, cardenolide and steroids	Ghamba *et al.*[63]
Cameroun and Nigeria	Variations in concentration of iron, phosphorous and calcium between sites	Ejoh *et al.*[24], Igile *et al.*[29] and Oshodi[72]

Table 4: Effect of phase development, cultivar and fertilizer application on chemical and bioactive compounds in *Vernonia amygdalina*

Factors	Key findings	References
Application of nitrogenous fertilizer	↑concentrations of cyanide, oxalate (soluble and total), vitamin C, nitrate and β-carotene	Musa *et al.*[73]
Reproductive stage	↑concentrations of cyanide, oxalate (soluble and total), vitamin C, nitrate and β-carotene. ↓mineral and protein content	Musa *et al.*[74] and Uzo and Mbachu[75]
Vegetative stage	↓cyanide, oxalate (soluble and total), vitamin C, nitrate and β-carotene. ↑mineral and protein content	Musa *et al.*[74] and Uzo and Mbachu[75]
Cultivar	↑protein content in purple shoot apex compared to others	Uzo and Mbachu[75]

flavonoids, alkaloids and cardiac glycosides were present. Oboh *et al.*[54] reported an absence of flavonoids and alkaloids in fresh VA leaves harvested from Edo State, South-South Nigeria while tannins, saponins and glycosides where present. Amodu *et al.*[30] reported the presence of alkaloids, flavonoids, tannins, cardiac glycosides, saponins, anthraquinones and anthracyanosides in VA leaves harvested from North Central Nigeria. Ndukwe *et al.*[27] reported the presence of phenols, flavonoids, tannins, saponins and steroids in VA leaves harvested from South Eastern Nigeria. Ghamba *et al.*[63] reported the absence of phenols and volatile oils in VA leaves harvested from North East Nigeria; while saponins, alkaloids, flavonoids, anthraquinones, tannins, terpenoids and steroids were present in various concentrations (Table 3). Ejoh *et al.*[24] found out that there is a variation in the value of iron, phosphorous and calcium from unprocessed VA leaves harvested from Cameroun compared to those harvested from Nigeria and attributed this to differences in cultivar, rainfall distribution, agricultural practice and location.

PHASE DEVELOPMENT, CULTIVAR AND FERTILIZER APPLICATION ON CHEMICAL AND BIOACTIVE COMPOUNDS

The literature on the effect of maturity on VA leaves is limited. However Musa *et al.*[73,74] studied the effect of reproductive phase on some micronutrients, anti-nutrients and toxic substances in VA. They found that the value of cyanide, oxalate (soluble and total), vitamin C, nitrate and β-carotene were higher in the leaves of VA treated with nitrogen-based fertilizers[74]. There were increases in the afore-mentioned compounds in the reproductive stage compared to vegetative stage of VA. However the mineral content of the leaves were higher in the vegetative stage which makes the leaf more edible as vegetable at the vegetative stage (Table 4). Uzo and Mbachu[75] identified six types of cultivars of VA and analyzed their protein content quarterly (January, March, June and October) as they grew. They found out the protein content increased as the growth progressed till June and dipped by October with purple shoot apex having the highest protein content.

CONCLUSION AND FUTURE PROSPECTS

Vernonia amygdalina (Del.) has been researched intensively in the last few decades for its usefulness in human and veterinary medicine. *Vernonia anygdalina* contains several bioactive compounds including phenols, tannins, flavonoids, saponins, anthraquinones and vitamins. These are known to have numerous medicinal and nutritional benefits. The leaves also contain resins, alpha- and iso-acids and essential oils and are valuable in beer breweries. Available evidence as described in this review, has shown that pre-harvest and post-harvest factors have substantial influence on the bioactive and chemical compounds of VA. Future research necessarily needs to illumine the effects of these different factors on specific phytochemicals in VA as that would lead to the derivation of maximal benefits (medically, economically, etc.) from *Vernonia amygdalina* (Del.).

Data from the available scientific literature are mostly limited to the general phytochemical screening of *Vernonia anygdalina*. It is important for studies to report specific information on the effect of pre-harvest and post-harvest factors on particular isolated and purified phytochemicals. Future studies should focus on the identification of specific

isolated and purified bioactive phytochemicals and the influence of pre-harvest and post-harvest factors on them. This will illumine the types of treatments that are compatible with deriving maximum beneficial effects from the bioactive compounds in the plant. The results of the recommended studies will also be relevant in optimizing postharvest handling and processing methods for *Vernonia amygdalina* leaves. These will not only advance the frontiers of knowledge but will also rob-off positively on the finances of the farmers, retailers, processors, in particular and the country in general.

SIGNIFICANCE STATEMENT

The review will help researchers to identify the best ways to treat materials from *Vernonia amygdalina* (Del.) in order to maximize the benefits derivable from any given phytochemical present in it. Thus a new theory on the role of pre-harvest and post-harvest factors in the biological activity of phytochemicals in *Vernonia amygdalina* (Del.) may be arrived at and used to optimize research and the development of products from the plant.

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