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# Proximate, mineral and phytochemical compositions of *Vernonia amygdalina* leaves grown under hot and humid tropical conditions

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**This study was conducted to determine the proximate and phytochemical compositions of *Vernonia amygdalina* leaves, to ascertain its nutritional potentials. *V. amygdalina* plants were cultivated, and the leaves were harvested at maturity, cleaned and apportioned into two; one portion was oven dried whilst the other was air-dried- all to a moisture content of about 15%. The dried samples were pulverized and used for proximate and phytochemical analysis. Data collected were subjected to two-sample T-test of GenStat Statistical Package (12th Edition). Results obtained show a significantly ($p < 0.05$) higher crude protein, dry matter, and ether extract (fat) contents in the oven-dried leaves, compared with the air-dried ones. The leaves had crude protein contents of about 25.32%, similar to those of commonly used feed ingredients, thus, implying that it can be used as feed for livestock. Phytochemicals present in the leaves include Flavonoids, Tannins, Saponins, Phenols and Terpenoids, and these could serve as therapeutic purposes in livestock species. The leaves however, had no alkaloids and glycosides, unlike similar plant species in Nigeria, thus, its use as livestock feed would pose no health threats to animals. It is recommended that *V. amygdalina* leaves should be used in livestock feeding trials, to document its effects on growth rates and disease resistance in livestock.**

**Key words:** *Vernonia amygdalina*, phytochemicals, mineral, crude protein contents.

## INTRODUCTION

Antibiotics and growth promoters have been widely used in livestock production as performance enhancers and for stress control, but most of these are synthetic in nature, and are perceived to leave residues in products of animals to which such substances have been administered (Nasir and Grashorn, 2008). Moreover, these synthetic ingredients are quite expensive, and their continuous use in livestock production has been reported

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to induce microbial resistance in livestock and in humans (Michard, 2008). Consequently, some consumers are refusing to patronize products of animals to which synthetic antibiotics have been administered. Various health organisations have put restrictions on use of synthetic antibiotics and growth promoters used in livestock production, due to the overwhelming rates at which microbes are developing resistance to antibiotics (Nasir and Grashorn, 2008).

The use of plants and plant parts in place of synthetic vaccines, has been widely accepted as a source of prophylactic and chemo-preventive drugs in livestock production (Soobrattee et al., 2006). According to Edeoga et al. (2005), utilisation of such plants to prevent and control livestock diseases has been found to be effective, affordable and readily available. Natural medicinal products, mostly of plant origin have been widely used in livestock production, with proven bioactivities such as antimicrobial, immune enhancement and stress reduction properties (Wang et al., 1998). Growth enhancement activities (Citarasu et al., 2002; Sivaram et al., 2004) and pharmacological screening of some medicinal plants have been tested as antimicrobial and nutrient supplements in poultry production (Thakare, 2004). Local farmers in Ghana have been using the leaves of *Vernonia amygdalina* as feed for livestock, and reported improvement in growth rates and disease resistance in the animals, but there is no documented evidence to that effect.

*V. amygdalina*, commonly called bitter leaf in Ghana, is a shrub that grows up to 3 m high in the African tropics, under a range of ecological zones to produce a large mass of forage (Bonsi et al., 1995; Igile et al., 1995). It is locally used as a remedy for skin infections, stomach aches, diabetes and for treating general weaknesses (Kupchan et al., 1969; Adodo, 2004). Okeke et al. (2015) conducted studies on the proximate and phytochemical composition of *V. amygdalina* plants in Nigeria, but there is little documented information on the proximate and phytochemical compositions of this plant, in Ghana. In addition, the earlier studies did not evaluate the mineral composition of the plant. This study was therefore conducted to determine the proximate, mineral and phytochemical compositions of *V. amygdalina* leaves in Ghana, to predict its nutritional and antimicrobial potential when used as feed for livestock.

## MATERIALS AND METHODS

### Experimental site

This study was conducted at the Laboratories of the School of Agriculture, University of Cape Coast, Cape Coast, Ghana. The area experiences a bimodal rainfall regime with a mean annual rainfall of about 920 mm. The temperature of the area is relatively high, with mean annual temperature of 23°C. The relative humidity is generally high (about 90%) in the night, but decreases gradually to about 70% in the afternoon when temperatures are high.

### Acquisition and processing of *V. amygdalina* leaves

Stem cuttings of *V. amygdalina* plant were cultivated at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast. At about 8 weeks after planting, the mature leaves were harvested, washed and apportioned into two; one portion was air-dried in shade at temperatures of about 26°C till moisture content of about 15% was attained. The second portion was oven-dried at a temperature of 50°C till moisture content of about 15% was attained. The dried leaves were pulverized, milled with a hammer mill, and were stored in air-tight transparent polyethene bags for proximate and phytochemical analyses.

### Proximate composition of the leaves

The crude protein content was determined by the Kjeldahl method, ether extract by the Soxhlet method, moisture and ash contents, all according to the methods described by the Association of Official Analytical Chemists (AOAC, 2000).

### Mineral analyses

Total Calcium, Magnesium, Phosphorus, Sodium, Potassium, Iron, Copper and Zinc contents of the *V. amygdalina* leaves were determined in this study. The Iron, Copper and Zinc contents were determined using Atomic Absorption Spectrophotometer (AAS), whilst the Potassium and Sodium levels were determined using Flame Photometer. The procedures outlined by the AOAC (2000) were followed in the analyses, and all analyses were conducted in triplicate.

### Phytochemical screening of *V. amygdalina* leaves

A mass of 300 g of the powdered sample was soaked in 1400 ml of distilled water and kept for 72 h. It was then filtered first using a white nylon cloth and subsequently using Whatman's No 1 filter paper, to obtain a dark-brown filtrate, which was concentrated over a hot water bath at 60°C and dried in a hot-air oven at 60°C to obtain the crude extract. The crude extract obtained was screened to identify the phytoconstituents present using standard phytochemical methods described by Sofowora (1993).

### Data analyses

All analyses and measurements were conducted in triplicates. Data obtained for the proximate, minerals and phytochemical constituents (quantitative) of the leaves, were subjected to the two-sample t-test component of the GenStat Statistical Package, 12$^{th}$ Edition (GenStat, 2012).

## RESULTS AND DISCUSSION

The proximate composition of the air and oven-dried samples of *V. amygdalina* leaves are presented in Table 1. The oven-dried samples had significantly ($p < 0.001$) higher crude protein, dry matter and ether extract contents than the air-dried samples. The air-dried samples, however, had higher ash content, than the oven-dried samples. That notwithstanding, the crude fibre content did not vary significantly ($p > 0.05$) between the

**Table 1.** Proximate composition of dried *V. amygdalina* leaves.

| Parameter (%) | ADL | ODL | SED | Sig. Level |
|---|---|---|---|---|
| Dry matter | 87.40[b] | 89.82[a] | 0.08 | *** |
| Crude protein | 22.67[b] | 25.32[a] | 0.26 | *** |
| Ash | 12.30[a] | 10.06[b] | 0.18 | *** |
| Crude fibre | 10.40 | 11.03 | 0.23 | NS |
| Ether extract (Fat) | 1.73[b] | 3.75[a] | 0.16 | *** |

Means in row with different letter superscripts are significantly different; SED – Standard Error of Difference; NS - Not Significantly different (p>0.05); ***- significantly different (p<0.001); ADL - Air-dried leaves; ODL - Oven-dried leaves.

treatments. The dry matter content of the leaves was quite high, ranging between 87.40 to 89.82%. It was realised that the drying methods applied, had significant ($p < 0.05$) effects on the proximate composition of the leaves.

The dry matter content was higher in the oven-dried leaves than in the air-dried ones. Although the dry matter contents varied significantly ($p < 0.001$) between treatments, the values recorded from this study, were higher than those reported by Oboh and Masodje (2009), but were quite lower than the 90.68 and 92.10% reported by Usunobun and Okolie (2016) and Belewu et al. (2009) respectively. Differences in dry matter contents observed between this and other studies, could be due to differences in the level of maturity of the leaves used, as older leaves are reported to have higher dry matter contents, than relatively younger ones. The magnitude of the dry matter content of the oven-dried samples in this study, is similar to findings of Ajayi et al. (2017), which reported that conventional ovens were more efficient in drying products, compared with other drying methods. This was explained to be due to the more consistent heat produced from the oven, compared with the air-drying method, which is highly influenced by the climate of the drying environment (Bankole et al., 2005).

Contrary to expectations, the oven-dried samples had significantly higher ($p < 0.05$) crude protein content, than the air-dried samples. Earlier research findings indicate that temperatures below 50°C do not adequately support efficient drying of substances (Nobrega et al., 2015), and thus, a longer duration would be required to properly dry the samples to appreciably low moisture contents. Meanwhile, other reports indicate that when oven-drying method is applied to biological materials, elevated temperatures or lengthy drying durations associated with this method, may cause adverse effects on the flavour, colour, and nutrients of the products (Qing-Guo et al., 2006). This is confirmed by reports of Schieber et al. (2001) which stated that temperature and duration of drying could contribute to degradation of volatile nutrients in products. That notwithstanding, the crude protein contents recorded in this study were similar to those reported by Usunobun and Okolie (2016). The observed trend among moisture and crude protein contents, however, corresponded with an earlier report of Aduku and Olukosi (2000), stating that moisture and protein contents are inversely related, thus, as protein content increases moisture content decreases, and vice versa. In general, it was realised that the crude protein levels of leaves used in this study, were higher than protein levels of most forage species used in feeding livestock, especially when they are harvested in the dry season.

The fibre contents were not significantly affected by the drying methods used. Fibre in livestock ration, tends to increase bulk, and reduces feed transit time in the alimentary canal, and incidence of constipation and related diseases (Ifon et al., 2009). Fibre is useful for maintaining motility, curing of nutritional disorders and digestion of food. Fibre level exceeding 20% in diets of non-ruminant livestock however, is associated with decreased nutrient utilization and low net energy values (Noblet and Le Goff, 2001). In the present study, the fibre levels of 10.40 and 11.03% recorded for air-dried and oven-dried samples respectively, were below the maximum threshold of 20% recommended by the National Research Council (NRC, 1994) for non-ruminant livestock species, hence, *V. amygdalina* could be used as feed for all livestock species.

## Mineral profile of *V. amygdalina* leaves

The mineral profile of *V. amygdalina* leaves is presented in Table 2. The Phosphorus, Potassium and Sodium contents of the dried leaves did not vary significantly ($p > 0.05$) among treatments. However, the Iron, Copper, Zinc, Calcium and Magnesium contents were significantly ($p < 0.01$) higher in the air-dried samples, than in the oven-dried ones. The Iron and Calcium levels were up to 768.2 and 27.704 µg per gram leaf sample respectively. It is observed that the values for Iron, Calcium and that of Magnesium were higher than the levels recommended by the NRC (1994). The plant contains sufficient amount of maximum level of daily intake of minerals for animals that feed on it. However, this does not guarantee absolute safety, since no test was conducted to ascertain the

**Table 2.** Mineral composition of dried *V. amygdalina* leaves.

| Parameter (µg/g) | MTL | ADL | ODL | SED | Sig. Level |
|---|---|---|---|---|---|
| Phosphorus | 10000 | 3547.00 | 3757.00 | 68.19 | NS |
| Potassium | 20000 | 17926.00 | 18274.00 | 414.00 | NS |
| Sodium | 45000 | 7438.00 | 7235.00 | 82.08 | NS |
| Iron | 500 | 768.20$^{a}$ | 642.40$^{b}$ | 9.04 | *** |
| Copper | 250 | 226.60$^{a}$ | 150.00$^{b}$ | 4.03 | *** |
| Zinc | 500 | 117.20$^{b}$ | 140.70$^{a}$ | 7.70 | * |
| Calcium | 15000 | 27704.00$^{a}$ | 20051.00$^{b}$ | 436.60 | *** |
| Magnesium | 6000 | 14062.00$^{a}$ | 9877.00$^{b}$ | 359.60 | *** |

Means in same row with different superscripts are significantly different; SED – Standard Error of Difference; NS – Not Significantly (p>0.05) different; * - Significant (p<0.05); *** - Significant (p<0.001); Sig - Significance; ADL – Air-dried leaves; ODL – Oven-dried leaves; MTL – Maximum Tolerable Level (prescribed by the NRC for poultry, swine, cattle and sheep).

**Table 3.** Phytochemicals present in dried leaves of *V. amygdalina* plant.

| Phytochemical | Types of leaf | |
|---|---|---|
| | Air-dried | Oven-dried |
| Terpenoids | + | + |
| Flavonoids | + | + |
| Alkaloids | - | - |
| Glycosides | - | - |
| Carotenoids | - | - |
| Tannins | + | + |
| Saponins | + | + |
| Anthraquinone | + | + |
| Phenols | + | + |

Key: + = Present; - = Absent

toxicity levels. Meanwhile, Calcium (Ca) is a major mineral component of skeleton of animals, thus, it is needed in adequate quantities for such purposes.

Iron (Fe), Zinc (Zn) and Magnesium (Mg) play important roles in metabolism of both plants and animals. Magnesium, for example, is required for proper functioning of enzymes and nervous system as well as for efficient carbohydrate metabolism. Deficiency of these micronutrients, therefore, has a direct effect on animal growth and metabolism (Zargar et al., 2015). High level of iron was recorded in this study. This is advantageous because iron plays a significant role in blood formation, and helps minimize incidence of anaemia in livestock (NRC, 1994).

The composition of these nutrients in *V. amygdalina* presents the plant as a potential growth enhancer. These, coupled with the high crude protein levels could have contributed to the improved growth rates reported by farmers who feed their livestock with the leaves of *V. amygdalina* plant.

## Phytochemical profile of dried *V. amygdalina* leaves

The phytochemicals present, and their levels in the dried *V. amygdalina* leaves are presented in Tables 3 and 4 respectively. Six out of the nine phytochemicals tested for, were present in the dried leaves. Terpenoids, flavonoids, tannins, saponins, anthraquinones and phenols tested positive, whereas alkaloids, glycosides and carotenoids were not detected in the samples. Findings from this study contradict those of Okeke et al. (2015), who detected alkaloids in *V. amygdalina* plants from Nigeria. The phytochemicals detected in this study are reported to play significant therapeutic roles, as well as growth enhancing roles in the body of animals (Valenzuela-Grijalva et al., 2017). It was realised that the levels of the phytochemicals detected, were significantly ($p < 0.05$) higher in the oven-dried samples, than in the air-dried samples; an indication that oven-drying at 50°C, is more efficient at retaining higher levels of phytochemicals, than air-drying the leaves in shade at 26°C.

The use of antibiotics and growth promoters in rearing meat animals, has been restricted in several countries, thus, increased interest in organic substitutes for such purposes (Put and Pasture, 2017). Dietary supplementation with secondary plant metabolites, referred to as phytochemical feed additives, is one of the alternatives proposed; thus, the use of phytochemicals in animal feed is accepted by consumers, just as herbal medicines are utilised by many patients. These phytochemicals have not only been proposed as replacements for antibiotics and growth promoters, but are also used for other anabolic compounds that promote growth in animals (Herrera et al., 2011; Devi et al., 2015). This indicates that phytochemicals have great prospects in livestock production, hence the use of *V. amygdalina* leaves as feed for livestock, could play significant roles in that regard.

Phytochemicals are significant sources of variety of

**Table 4.** Levels of phytochemicals present in the leaves of *V. amygdalina* plant.

| Parameter (mg/L) | ADL | ODL | SED | Sig. Level |
|---|---|---|---|---|
| Flavonoids | 0.80 | 4.13 | 0.004 | *** |
| Tannins | 27.90 | 67.99 | 0.001 | *** |
| Saponins | 30.14 | 72.12 | 0.002 | *** |
| Phenols | 0.76 | 1.64 | 0.002 | *** |
| Terpenoids | 0.61 | 1.56 | 0.003 | *** |

***** -** $p < 0.001$; SED – Standard Error of Difference; Sig. – Significance; ADL – Air-dried leaves; ODL – Oven-dried leaves; Means in row with different letter superscripts are significantly different ($p < 0.001$).

compounds with different biological activities including antimicrobial, antioxidant, anti-stress, and nutrigenomic effects on the development of immunity that have made them attractive for use as growth promoters in animal production. Flavonoids are polyphenolic compounds called “functional ingredients” and “health promoting biomolecules” due to their potential role in promoting health and preventing chronic degenerative diseases (Nijveldt et al., 2001). Flavonoids have been shown to possess anti-inflammatory, antioxidant, antibacterial, antiviral, hepatoprotective, antiallergic, antithrombotic, anticarcinogenic and immunomodulator activities in a number of *in-vitro* and animal model studies (Cushnie and Lamb, 2005).

Saponin is one of the secondary components found in tropical plants that have been reported to perform various roles in animal production (Cheeke, 2009; Wina, 2012). Saponin is significantly used as an adjuvant in several vaccines to increase immunogenicity of an antigen, resulting in increased antibodies and cytotoxic of T-lymphocytes to improve the efficacy of vaccines (Wina et al., 2018). From earlier studies, mixture of saponin-containing plants enriched with polyphenol, improved weight gain in animals as compared to those treated with antibiotics and growth promoters; suggesting that saponin mixture could be used as a substitute for antibiotics and growth promoters in livestock production (Cheeke, 2009). Plant extracts containing saponin have also been reported to have antifungal property, antibacterial and high antioxidant activities (Zhang and Zhou, 2013). Animals would likely get all these benefits, if they are fed with leaves of *V. amygdalina* plant.

It can be recalled from Table 3, that alkaloids, glycosides and carotenoids were not identified in both the air-dried and oven-dried leaves of *V. amygdalina*, contradicting findings of Okeke et al. (2015), who identified alkaloids in *V. amygdalina* from Nigeria. This indicates that location of the plant could affect its phytochemical composition. The absence of alkaloids in the leaves of *V. amygdalina* plants from Ghana, is good news because alkaloids and glycosides are reported to have negative impacts on growth of livestock, hence, their unavailability indicates that the plant, if fed to animals would not pose health threats to them.

## CONCLUSION AND RECOMMENDATIONS

*V. amygdalina* leaves have crude protein contents of up to 25%, and fibre content of about 13%; thus, it could be used as feed for livestock. Oven dried leaves at 50°C had higher crude protein, ether extract and most of the minerals screened for, as compared with samples dried in shade at 26°C. The plant has high levels of Iron and Calcium; thus, its use as livestock feed can help minimize incidence of anaemia, and improve bone and teeth development in animals. Phytochemicals present in the leaves of *V. amygdalina* include Flavonoids, Tannins, Saponins, Phenols and Terpenoids; indicating that the leaves can play antimicrobial and growth promoter roles if fed to livestock. The findings from the present studies indicate that *V. amygdalina* leaves have potential for use as feed material for livestock, for possible improvement in growth and disease resistance in the animals. However, this study did not ascertain the toxicity level of the plants. It is therefore recommended that *V. amygdalina* leaves should be subjected to toxicity studies and used in livestock feeding trials, to document its effects on growth rates and disease resistance in livestock.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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