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Determination of Proximate, Phytochemicals and Minerals Composition of *Vernonia amygdalina* (Bitter Leaf)

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ABSTRACT

Vegetables are the major sources of vitamins, minerals and bioactive components such as phytochemicals and antioxidants that help in reducing the risk of diseases. The aim of the study was to determine the proximate composition, phytochemical screening and mineral composition of Vernonia amygdalina leaf extract. The proximate composition and phytochemical screenings were determined by using conventional laboratory methods, while the mineral composition of the leaves was determined by using atomic absorption spectrophotometer. The proximate analysis of the leaf extract showed that, it contains carbohydrates (37%), proteins (28.2%), fats (5.5%), crude fiber (11.6%), moisture content (8.4%) and ash content (9.3%). The preliminary phytochemical screening of *V. amygdalina* leaf extract revealed the presence of alkaloids, terpenoids, flavonoids, steroids, phenols, saponins, and tannins. Quantitative phytochemical analysis of the extract showed that the flavonoids are the most abundant constituent which is about 12.2%, followed by steroids, alkaloids, and phenols constituting 4.8%, 4.6%, and 3.6% respectively. The mineral analysis of the extract indicate the presence of calcium (61 mg/100g), potassium (61 mg/100g), magnesium (85.8 mg/100g), phosphorous (60.5 mg/100g), zinc (9 mg/100g), iron (15.2 mg/100g) and copper (5 mg/100g). From the findings of this study, it is concluded that *V. amygdalina* leaf has therapeutic potential and can be used in dietary supplements.

Keywords: Minerals, Phytochemicals, Proximate analysis, *Vernonia amygdalina*

INTRODUCTION

Several compounds such as vitamins, minerals and bioactive components like phytochemicals and antioxidants which help in reducing the risk of chronic illness of vegetables [1]. According to English dictionary, vegetables are the plants raised for its edible part, such as leaves, roots, seeds, and

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flowers. Leafy vegetables have high nutritional components on consumption they provide proteins, vitamins, minerals, and fiber for body. They remain an important edible food and easily available diet for human especially in the rural areas [2]. The analysis of bioactive components (such as phytochemical and anti-oxidant composition) of vegetables, encourage their utilization as natural medicine in pharmaceutical industry with nutraceutical values [3]. Vegetables are essential for the developing and under developed countries where vitamins and mineral supply is inadequate to meet the nutritional requirement for rapid growing population.

V. amygdalina (bitter leaf) belongs to the family Astreraceae which has a characteristic feature of bitterness in taste. It is a small shrub (Figure 1) that can grow up to three meters and native to tropical Africa. The plant is also well distributed in Asia and more commonly found near drainage line and in natural forests [4]. The leaves of the plant are used for making soup and stew in tropical Africa and majorly used ethno-botanically for the treatment of different ailments [5]. *V. amygdalina* is a medicinal plant used in folk medicine for treatment of several diseases. The bitter taste of *V. amygdalina* can be reduced by boiling or soaking in water [6]. Several bioactive alkaloid saponins and tannins are present in the plant leaves. These bioactive components made out of *V. amygdalina* leaf acts as an antimicrobial agent in brewing industries [7]. Several ailments such as fever, kidney problems, hiccups, and stomach discomfort can be treated ethno-medically using extracts of *V. amygdalina* leaves and roots [6]. *V. amygdalina* leaf and root extracts are used as purgative, antifungal and anti-malaria agents [8]. In pharmaceutical industry, *V. amygdalina* has acquired special relevance recently due to possession of anti-tumorigenic properties [9]. Nutritionally, *V. amygdalina* leaves are used as appetizer and also used in production of soap [10] and has been used as food supplement for weaning babies [11].

Several studies conducted on different parts of *V. amygdalina* indicated that it contains different bioactive components such as alkaloids, flavonoids, tannins, saponins, triterpenoids, and many more [12]. These bioactive components are responsible for their different pharmacological properties such as anti-malaria, antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, antithrombotic, laxative, anticancer, antihelmintic, and hypoglycemic among others [13,14]. Considering the nutritional values of *V. amygdalina*, the study was conducted to determine the proximate, phytochemical and mineral composition of *V. amygdalina* leaf extract.

Figure 1: *V. amygdalina* plant

METHODOLOGY

Collection and Identification of *V. Amygdalina* Leaves

The leaves of *V. amygdalina* were purchased from Sabon-Gari Market located in Kano Metropolis Kano, Nigeria. The plant species to which these leaves belonged were identified at Herbarium (Department of Plant Science, Bayero University Kano). A voucher number of BUKHAN 0235 was assigned to the specimens. Leaves were washed, air dried and pulverized into fine powder, then stored in air tight container for further use.

Extraction of the Plant Material

Twenty five grams (25g) of the powder was soaked in 250 mL conical flask containging distilled water [15] for 72 hours. Filtration method using filter paper was employed to separate the solution and the filtrate obtained was subjected to heat for dryness in 60°C temperature water bath. The solid extract was stored in sterile universal bottles and refrigerated at 4°C for further use.

Determination of Proximate Composition

The proximate composition of *V. amygdalina* leaf was determined using standard laboratory method to determine carbohydrates, fats, ash contents, dry matters, crude proteins, and crude fibre [16]. The proximate parameters expressed in percentage were obtained by taking the average values twice in triplicates.

Phytochemical Screening

The preliminary phytochemical screening of *V. amygdalina* leaf extract was conducted using conventional methods described by Sofowora A, [17] and Trease GE and Evans WC, [18]. The method was employed to determine the presence phyto-constituents such as flavonoids, alkaloid, phenol, terpenoid, steroid, tannins and saponins.

Qualitative Phytochemical Analysis

Various methods were employed in determining the amount of bioactive components (phytochemicals) present in *V. amygdalina* leaf. Terpenoids, steroids, and tannins were determined using Spectrophotometric method while phenol was determined using Folin-Ciocalteu procedure. The alkaloids, flavonoids, and the content of saponins were evaluated using standard method described below;

Determination of Alkaloids

Alkaloids were determined using Haborne JB, method [19]. Briefly, 10g of *V. amygdalina* leaf powder was dispersed in 100 ml of 10 acetic acid solution in ethanol. The mixture was shaken thoroughly and allowed to stand for 5 hours, then filtered. Hot plate was used to evaporate the filtrate to about one quarter of its volume. Drops of concentrated ammonia were added to precipitate the alkaloid. An already weighted filter was used to filter off the precipitate, and then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was oven dried at 60°C for about 30 minutes. The filter was cooled in desiccator and re-weighted until a constant weight was obtained. Alkaloid was determined by weight difference in filter paper and expressed as percentage of the sample weighted was analyzed.

Determination of Flavonoid

Flavonoid determination was done as described by Ejikeme CM, et al. [20]. Exactly 50 cm^3 of 80% aqueous methanol added was added to 2.50g of sample in a 250 cm^3 beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper was used to filter whole solution of each wood sample. Each wood sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoids was calculated as;

$$\% \text{ of Flavonoid} = \frac{\text{Weight of flavonoid}}{\text{Weight of sample}} \times 100$$

Determination of Total Saponin

About 20g of *V. amygdalina* leaf extract was added into conical flask containing 100 mL of 20% ethanol. The sample was heated in water bath while shaking continuously for 4 hours at 55°C. The mixture is then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts are reduced to 40 ml over a water bath at about 90°C. The concentrate is then transferred into a 250 ml separating funnel and 20 ml of diethyl ether is added to the extract and vigorously shaken. The aqueous layer is recovered while the diethyl ether layer is discarded and the purification process is repeated. 60 ml of n-butanol is added and the combined n-butanol extracts is washed twice with 10 ml of 5% sodium chloride. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight and values are expressed as percentage of extract [19].

Determination of Mineral Composition

Atomic absorption spectrophotometer [16] was used to determine the mineral composition of the leaves such as calcium (K), zinc (Zn), potassium (K), iron (Fe) phosphorous (P) and magnesium (Mg). The result obtained was expressed mg/100g dried powder.

RESULTS

Proximate Composition

The result of proximate composition (both qualitative and quantitative) of *V. amygdalina* leaf is presented in table 1. The qualitative result showed the presence fats, carbohydrate, protein, fibre, ash and moisture. Quantitatively, carbohydrate has the highest composition which accounted for 37 mg/100g, this is followed by protein (28.2 mg/100g), crude fibre (11.6 mg/100g), and ash content (9.3 mg/100g) and moisture content 8.4 mg/100g while fats has the least composition which accounted for 5.5 mg/100g.

S/N	Nutrients	Composition (mg/100g)
1	Carbohydrate	37.00 ± 1.50
2	Protein	28.20 ± 1.20
3	Fats	5.50 ± 0.23
4	Crude fibre	11.60 ± 0.30
5	Moisture content	8.40 ± 0.04
6	Ash content	9.30 ± 0.23

Table 1: Proximate analysis of *V. amygdalina* leaf.

Phytochemical Screening

Table 2 represents the result of preliminary phytochemical screening of *V. amygdalina* leaf extract. Both qualitative and quantitative results were presented. The following phytochemicals were obtained; flavonoid, alkaloid, saponin, tannin, terpenoid, phenol and steroid. On the other hand, flavonoid has the highest content (12.2%), followed by steroid (4.8%), alkaloid (4.6%) and phenol (3.6%).

S/N	Phytochemicals	Qualitative screening (mg/100g)	Quantitative screening (%/100g)
1	Alkaloid	+	4.60±0.23
2	Flavonoid	+	12.20±1.30
3	Saponin	+	2.70±0.5 0
4	Steroids	+	4.80±0.25
5	Terpenoid	+	1.70±0.04
6	Phenol	+	3.60±0.20
7	Tannin	+	1.20±0.03

Table 2: Qualitative and quantitative phytochemical screening of *V. amygdalina* leaf extract. Key: + = Present, - = absent of phytochemical.

Mineral Analysis

Table 3 represent the mineral analysis of *V. amygdalina* leaf. The result in mg/100mg indicated that magnesium has the highest composition (61mg/100g), followed by calcium (65.5mg/100g), potassium (61mg/100g) and phosphorous (60.5mg/100g). others include; iron (15.2mg/100g), zinc (9mg/100g) and copper (5.3mg/100g).

S/N	Minerals	Composition (mg/100g)
1	Potassium	61.00
2	Calcium	65.50
3	Magnesium	85.80
4	Phosphorous	60.50
5	Zinc	9.00
6	Iron	15.20
7	Copper	5.30

Table 3: Mineral analysis of *V. amygdalina* leaf.

DISCUSSION

The preliminary phytochemical screening of *V. amygdalina* leaves extract revealed the presence of saponins, flavonoids, tannins, terpenoids, alkaloids, phenols, and steroids. These bioactive components are beneficial to human health exhibiting different biochemical and pharmacological actions as well as possessing antioxidant activity [21]. Several studies were conducted to determine and characterized various bioactive components of *V. amygdalina* leaf extracts [7,22-24]. This resulted in screening of various bioactive components such as alkaloids, flavonoids, tannins, saponins, and phenolics [13,25]. Findings from the present study correlate with those of Atangwho IJ, et al. [26] and Ndukwe OK, et al. [27].

Alkaloids play an important metabolic roles and development in the system of living organisms [28]. It is beneficial chemical to plants serving as repellent to parasites and predators. The alkaloid is known to contain antimicrobial agents which accounted for its antimicrobial activity [3]. Flavonoid is believed to contain antioxidant agents and it is reported that it reduce the oxidation of low-density lipoprotein, lower cholesterol level and triglyceride [29]. It is also expressed in plant in respond to microbial attack suggesting their antimicrobial property [30]. Saponins limit the growth and viability of cancer cell by reacting with cholesterol rich membrane of cancer cell [31]. Pharmacologically, saponin is responsible for most cellular activities related to cell division and growth in human and has incivility effect on inflammation. Hence, *V. amygdalina* leaves can be utilized for the management of inflammation [32,33]. Steroid is important pharmaceutically for production of drugs due to possession of compound showing similarities to sex hormones [34]. Terpenoids are known to possess anticancer, anti-parasitic, antimicrobial, antifungal, immunomodulatory, anti-inflammatory, antiviral, anti-allergic and antispasmodic

properties [35]. Phenolics are reported to possessed antioxidant property which prevents oxidative damage of cell due to present of free radical scavengers [36]. The phenolics lower the risk of heart diseases and provide anti-inflammatory activity due to their ability to neutralize or scavenge free radicals [37]. Tannins are known to have potential antiviral activity [38] as well as anticancer agent [39].

The proximate composition of V. amygdalina leaf according to the present study contains a high amount of protein and carbohydrate. However, there are moderate amount of fiber, ash, moisture and little amount of fat. This result supported the findings of Owu D, et al. [40] that *V. amygdalina* contains carbohydrate, protein, fiber, fats, amino acid, minerals and vitamins. High content of carbohydrate (37%) in *V. amygdalina* leaf from this study indicated that it is a good source of energy [3]. Presence of protein in V. amygdalina is very vital. The proteins are building blocks need to built-up hormones, enzymes, brain chemicals, and nucleic acids. Antibodies produced by protein are used to fight against germs [41]. From the result of the present study, *V. amygdalina* leaf contains crude fiber of about 11.6% and this justified the work of Yeap SK, et al. [42] who reported that *V. amygdalina* leaves contained 6.5 to 29.2% of crude fiber. The fiber inhibits the intake of starchy food and hence, prevents body metabolic condition such as diabetes and cholesterol [43]. According to the present study, *V. amygdalina* contain low fat (5.5%). This agrees with the report of Yeap SK, et al. [42] who reported crude fat values ranging from 2–15%. The food providing 1–2% of fat is sufficient for healthy human as excess fat consumption has implication and may lead to certain cardio-vascular disorder [44].

Analysis of mineral composition of V. amygdalina leaf in this study confirmed the presence of both trace (zinc, iron and copper) and major (potassium, phosphorous, calcium and magnesium) elements. This justifies the vitality of the plant leaf nutritionally rich when consumed by animals or humans. Minerals play an important metabolic role in the body of animals, such activities include maintenance of acid balance in the body, production and activity of enzymes and so on [3]. Presence of potassium in the extracellular body fluid is vital; it conducts several functions to the body system such as regulation of osmotic pressure, conduction of nerve impulse and maintenance of acid-base balance [3]. Calcium played a major role in the formation and development of bones and teeth, coagulation of blood, contraction of muscle, normal functioning of heart and nervous system [45]. Presence of magnesium in the diet is essential for decreasing blood sugar as a result it improves the function of insulin [46], metabolism of fats, and carbohydrates [3]. Presence of zinc in *V. amygdalina* leaf made it important for nerve functioning and normal sexual development. Zinc is also vital for stimulating the activity of vitamins as well as formation of red and white blood cells [47]. Zinc is an integral part of many enzymes in the body and also plays an important role in proper functioning of body immunity [46]. Copper as a trace element is essential for cellular defense, mucous membrane protection, anti-anemic and vital for haemoglobin formation [47]. Despite the nutraceutical value of *V. amygdalina*, the plant can be toxic due to the unrefined nature of the preparation or lack of specificity in the application of the plant which could lead to over dosage of the herbal preparation. This may result in accumulation of toxic plant ingredient in the human system. A study conducted by Okwuzu JO, et al. [48] on cytotoxicity testing of aqueous extract of *V. amygdalina* showed high cytotoxic effects induced by *V. amygdalina* extract.

CONCLUSION

Based on the findings of the present study, the *V. amygdalina* leaf extract contains an adequate amount of food substances, phytochemicals and mineral elements and thus provide a basic rationale for the use of the plant as herbal medicine and food substances.

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