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# Phenolic content, flavonoid content and antioxidant properties of *Vernonia amygdalina* butanol leaf extract. (Bitter leaf)

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**Abstract**
*Vernonia amygdalina* also known as bitter leaf is a member of the asteraceace family. It is used in the traditional treatment of various diseases in Africa. This study appraises the antioxidant activities, phenolic and flavonoid phytochemical properties of the leave. In addition to the estimation of the total phenol content, flavonoid content and ascorbic acid content. The activity of the extract was evaluated by measuring the scavenging activity which shows mean percentage of 50.74% at 0.3mg/ml, the reducing power is 41.74% at 0.3mg/ml and the percentage composition of flavonoid 1.273%, phenol 0.381% and vitamin c (ascorbic acid) 48.40mg per 100g. The presence of these antioxidatives parameters in the leaves extract showed the ability of the leaves to prevent free radicals which causes damage or death of cells. The result of this work showed that the leave extract can be utilized as an effective and safe antioxidant source.

**Keywords:** *Vernonia amygdalina*, antioxidant, phenol, flavonoid, radicals

## Introduction

There has been a great deal of interest recently in the role of complementary and alternative medicine for the treatment of various acute and chronic diseases (Aruoma, 2006) [3] of the various classes of phytochemicals. Interest has focused on the anti-inflammatory and antioxidant properties of polyphenols found in various botanical agents. Plants vegetables and species used in folk and traditional medicine has gained wide acceptance as one of the main source of prophylactic and chemopreventive drug discovery and development (Aruoma, 2006) [3]. Medicinal plants constitute the main source of new Pharmaceutical and healthcare products; they have been documented for their beneficial properties that can protect human against diseases, (Kumar *et al*., 2009) [8]. It had been known that they contain certain compounds and micronutrients used as an anti-inflammatory, Antioxidant, antibacterial, analgesic and anti-anemic drugs. Medicinal plants are of much interest due to their antioxidants and free radical scavenging properties. These antioxidants are electron-deficient compounds with the readiness to donate electron to electron deficient compounds such as free radicals to stabilize their actions. Free radicals are electron-deficient compound because they possess unpaired valance electrons (Kadam *et al*., 2010; Aluko *et al*., 2013) [7, 1] and in their quest to finding electrons, they can easily attack cells and biomolecules in the body resulting in generation of diseases. Several studies has linked the generation of reactive oxygen species (free radicals) such as hydroxyl (OH) radical, superoxide ($O_{2)}$, nitric oxide (NOA), nitrogen dioxide ($NO_2$), peroxyl (ROO), hydrogen peroxide ($H_2O_2$) to the development of pathological conditions including protein oxidation, lipid peroxidation, DNA damage and cellular degeneration of these conditions have been implicated in the actiology of disease such as diabetes, cancer, Alzheimer and Parkinson disease, cardiovascular disease, ageing process, arthritis and inflammation (Aruoma, 2003) [2].

Free radicals have been implicated in the development of a number of disorders, including cancer, neurodegeneration and inflammation, giving rise to studies of antioxidants for the prevention and treatment of diseases (Baba and Malik, 2015) [4]. Most of the drugs that are currently available on the market for the treatment of various serious diseases have limited potential because they are expensive and produce detectable side effects. Therefore, it necessary to find effective treatments for various disorders excluding the above-mentioned limitations of the marketed drugs. Fruits, vegetables, and herbal plants have been shown to be rich sources of chemicals with the potential to prevent incurable diseases (Al-Matani *et al*., 2015).

*Vernonia amygdalina* is traditionally used to treat a variety of diseases including diarrhoea, fungal and bacterial infections, inflammation, cancer, diabetes, and its squeezed juice can be applied on wounds (Ugbogu *et al*., 2021) [9]. *Vernonia amygdalina*, commonly known as bitter leaf is a stub that grows up to 3 meters high in the African tropics and other parts of Africa, particularly, Nigeria, Cameroon and Zimbabwe. It is reputed to have several health benefits. The presence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases. Although the plant is widely used in traditional medicine, few studies have been conducted of the pharmacological activities of the plant.

Phenolic and flavonoid compounds are widespread in plant kingdom where they act as antioxidants and free radical scavengers (Baba and Malik, 2015) [4]. The target plant of study contains bioactive constituents as reported in previous work literature. There is now an urgent need to search for novel and effective medicines in plants that may be the remedy for incurable diseases. Hence, the present study aimed at investigating the total phenolic content, total flavonoid content and antioxidant activity of bitter leaf.

**Materials and Method**

**Sample collection**

The freshly harvested *V. amygdalina* (bitter leaf) were purchased from Ekeonuwa market Owerri, Imo state and taxonomically identified by Dr. Duru, C.N. in the department of microbiology.

**Sample Preparation**

The bitter leaf was washed and dried at room temperature for three days. The dried sample were ground into powder using grounding machine and 30g of the sample was extracted with soxhlet extractor using methanol as the extracting solvent. The extract was concentrated by evaporating the methanol using a water bath. After which the extract was stored at 4$^{o}$c for use.

**Determination of Total Phenol**

The follins method described by peason (1976) was used 0.2g of the dried ground sample was dispersed in 10mls of methanol and shaken. The mixture was allowed to standard for 30mins at room temperature before it was filtered through what man filter paper exactly 1ml of the extract was place in a test tube and 1ml of follin reagent was added to it with 5mls of distilled water. The colour was allowed to develop for about 3-4 hours at room temperature. The absorbance of the develop colour was measured at 760nm wavelength. The procedure was repeated for 2 or more times to get an average. The phenol content was calculated, thus;

$$\% \text{ phenol} = \frac{100 \text{ x ay x cx vf x } \Delta}{\text{W a8 1000 v9}}$$

Where;
W = weight of sample analyzed
A4= absorbance of test sample
As= absorbance of standard solution
C= concentration of standard in mg/ml
VF= volume of total filtrate
V9= volume of filtrate analyzed
D= dilution factor where applicable

**Determination of Flavonoids**

This was determined gravimetrically using the method described by Harborne (1973). Exactly 5g of the sample was boiled in 100ml of 2m HCL solution for 30mins. The boiled mixture was allowed to cool and then filtered through Whatman No. 42 filter paper. The filtered was treated with ethyl acelete starting with drop wise addition until in excess. The precipitated flavonoid was recovered by filtration using a weighed filter paper and dried in an oven at 80c%, cooled in a dessicator and reweighed. The difference in weight gaze the weight of flavoniod which was expressed as a percentage of the weight analyzed. Given by the formulars below.

$$\% \text{ flavonoid} = \frac{\text{w2} - \text{w1 x } 100}{\text{w1}}$$

Where;
W= weight of sample
W1= weight of empty filter paper
W2= weight of filter paper + flavonoid precipitate.

**Determination of Chelating Potential**

**Metal (Ferrous ion) chelating activity**.

The chelating activity was determined by a method described by Ebrahimzadeh, *et al*., (2009). The ability of the extract to chelate ferrous ion ($Fe^{2+}$) was evaluated. 0.3ml concentration of the extract were prepared and 1ml of each concentration was mixed with 1ml of 0.25 $FeS_{o4}$ and 1ml of 0.3125m Ferozine and shaken vigorously. After incubating for 10 minutes at room temperature, the mixture solution was measured using a spectrophotometer at 56nm against a blank. Sodium EDTA was used control percentage inhibitor of ferozine ($Fe^{2+}$) by the extract was determined using the equation:

inhibition of ferrozine (%) as – au x 100
as1

AS= Absorbance of control
Au= Absorbance of sample

**Determination of the Reducing Power BY OYIZU (1986)**
The reducing power of the extract was determine according to the method of Oyizu (1986) 1ml of the extract with a volume of 0.3ml. 1ml of sodium phosphate buffer (0.2ml OH 6.6) and 1ml of potassium ferricyanide and (10mg/ml) were mixed and incubated at 50˚c for 20mins. Then 1ml of 10% TCA was added to the mixture and centrifuged at 13.400x g for 5mins. Then 1ml of g supernatant was mixed with 1ml of $H_20_2$ and 0.1ml of 0.1% ferric chloride and then the absorbance was measured at 700nm.
Reducing power = am

ab – 1 x 100

Where
AM= Absorbance of reaction mixture
AB = Absorbance of control mixture.

**DPPH Radical Scavenging**
Methalonic solution of the extract was mixed with 400nm DPPH (Sigma Aldrich) methanol solution at a ratio 1; 3. The mixture was left in dark at room temperature for 90mins. The absorbance of the resulting solution was measured by spectrophotometer (Srimadzed 1700) at 517nm. The capability of scavenging DPPH radical was then calculated by adding the following equation:

Scavenging effect (%) = (1 – Abs. of sample / abs of control) x 100

**Determination of vitamin C.**
The barakat titrimetric method was used. A measured volume (5g) of the sample was extracted with 100ml of the mixed extracted solution 6% tri-chloroactic acid/ethylene di-aminetetra acetic acid. Allow to stand for 30mins, after 30min, mixed at room temperature, it was filtered and the filtrate. (I.E the extract) was used for the analysis. An aliquote of the extract (20ml) was dispatched into a conical flask and treated 10ml of 30%KI solution followed by 100ml of distilled water. It was filtered against 0.01 mole of cooper sulphate (cuso4 solution) using 1% starch solution as indicator. Then end point was measured by the presence of dark specks at the brick of the flask. The vitamin C content was calculated using the formular below;
1ml of 0.01m CUSO4 = 0.88mg of vitamin C

$$\text{Vitamin C mg/100g} = 100g = \frac{100}{w} \times 0.88 \times \text{titre} \times \frac{vt}{vg}$$

Where;
W=Weight of sample
VF= Total volume of extract
VA= Volume of extract tittered.

**Results**
The result obtained during the investigation was produced and analyzed below

**Table 1:** Bioactive components of *Vernonia amygdalina*

| Parameters | Value |
|---|---|
| Phenol (%) | 0.381 |
| Flavonoid (%) | 1.273 |
| Vitamin c (mg/100) | 48.40 |

Antioxidant scavenging activity of of *Vernonia amygdalina*

**Table 2:** Antioxidant Potential

| Parameter | % Inhibitions | Standard Vit. C (%) |
|---|---|---|
| DPPH | 46.04 ± 0.03 | 60.80 |
| Reducing Potential | 41.74 ± 0.02 | 60.80 |

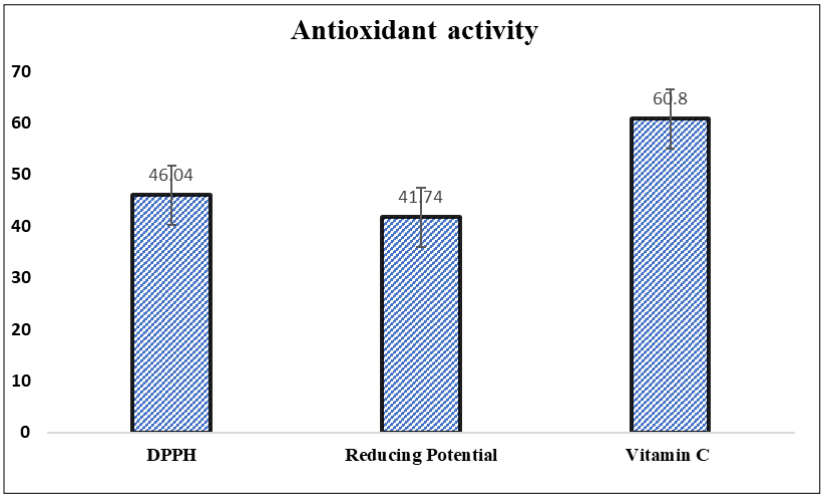


**Fig 1:** Antioxidative potentials of *Vernonia amygdalina*

## Discussion

*V. amygdalina* has several medical, industrial, food, and traditional uses. The plant is used as a tonic in the treatment of fever, constipation, and many illnesses in traditional and herbal Nigerian medicine (Howard *et al*., 2016) [6]. Our study investigated the antioxidantive properties of *vernonia amygadalin*. The biomolecules determination was represented in table one. The phenol content of *vernonia amygdalina* leaf was found to be 0.381%. The flavoniod content of the leaf was 1.273%, the ascorbic content of the leaf was 48.40. It is well known that plant phenolics are highly free radical terminators (scavengers) antioxidant. Antioxidative activities of vegetable and fruits are derived from phenol, flavonoid, ascorbic acid, authocyanin in this leaf extract was appreciable.

The reducing power is used as the absolute measure of antioxidants present in the extract caused the reducing power 41.74% 0.3mg/ml concentration. The antioxidants present in the extract caused the reduction of Fe 3+ ferriccyanide complex to the ferrous form Fe 2+ with a change in colour from yellow to bluish green (lou *et al*., 2006) and proved the reducing ability power ability. The result of the leaf extract showed that it has a good free radical scavenger. Table 2 shows that the mean value for DPPH radical scavenging activity at 0.3mg/ml concentration is 46.04%. Free radical are known to be major culprit in most degenerated disease like diabetes, Alzheimer, hypertension, Parkinson's Cardiovascular disease and cancer. For the plant to be good candidate for trail in the search for good drug against disease associated with oxidative stress. Our study was in-line with Erasto *et al*. (2006) [5] which investigated the antioxidative activity of acetone, methanol and water extracts of *V. amygdalina*. The antioxidative activity of the extract was determined by detecting the reduction of the absorbance of DPPH at 519nm. Results showed methanol extracts with highest antioxidative activity compared to the acetone and water extract. Methanolic extracts have antioxidative activity by scavenging 75.9%, 93.9%, 97.1%, and 99.3% of the DPPH radicals from 0.01, 0.02, 0.05, and 0.1 mg/ml of extracts. Acetone extracts scavenged radicals between 63.3% and 91.7%. Results from this study elucidated the antioxidative activity of *V. amygdalina*

## Conclusion

The leaves of *Vernonia amygdalina* (Bitter leaf) from the data carried out, reveals that they contain an appreciable amount of polyphenol and antioxidants. And having seen that the leaves contains high amount of both phytochemicals like flavonoids, vitamin c and significant scavenging properties/potentials, it was concluded that the leaves is very beneficial for both consumption as nutritional vegetables and medicinal plants. Further studies should be carried out on both the leaves and stem of *Vernonia amygdalina*. Pharmaceutical industries should also use this plant leaves for drugs formulation.

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