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EVALUATION OF THE ANTIOXIDANT POTENTIALS OF *Vernonia amygdalina* (BITTER LEAVES)

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Abstract
Vernonia amygdalina (bitter leaf) is a widely used medicinal plant in Africa, traditionally valued for its therapeutic benefits, including the management of oxidative stress-related conditions. This study investigated the antioxidant potentials of *Vernonia amygdalina* (bitter leaf) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric-Reducing Antioxidant Power (FRAP) assays. Fresh bitter leaves were collected, authenticated, dried, pulverized, and extracted with methanol using cold maceration. The antioxidant activity was evaluated at concentrations of 10–50 µg/ml, and the inhibitory concentration 50 (IC_{50}) and effective concentration 50 (EC_{50}) values were determined from inhibition–concentration curves. The results showed that bitter leaf extract exhibited significant antioxidant activity, with a low IC_{50} value of 8.13 µg/ml in the DPPH assay, indicating high free radical scavenging ability. The extract also demonstrated a moderate EC_{50} value of 24.31 µg/ml in the FRAP assay, suggesting moderate reducing power. These findings suggest that *Vernonia amygdalina* may be a valuable source of natural antioxidants, with potential applications in the prevention and treatment of oxidative stress-related diseases. The study highlights the need for further research into the bioactive compounds and mechanisms underlying the antioxidant properties of bitter leaf.

INTRODUCTION
Reactive oxygen species (ROS) are unstable molecules that contain oxygen and react easily with other molecules in a cell, causing oxidative stress and associated diseases (Halliwell, 2007). Oxidative stress is a state of imbalance between the production of ROS and the body's ability to neutralize them, leading to cell damage and contributing to various diseases, including cancer, diabetes, and cardiovascular disease (Valko et al., 2007).

Antioxidants are substances that can neutralize or mop up ROS, thereby preventing oxidative stress and its associated diseases (Carocho and Ferreira, 2013). Antioxidants can be classified into two main categories: natural and synthetic. Natural antioxidants are derived from plants and include vitamins C and E, beta-carotene, and polyphenols, while synthetic antioxidants are man-made and include butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Larson, 1988).

In recent years, there has been a growing interest in the use of natural antioxidants, particularly those derived from plants, due to their potential health benefits and lower toxicity compared to synthetic antioxidants (Aremu et al., 2011). One such plant is *Vernonia amygdalina*, commonly known as bitter leaves.

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Vernonia amygdalina is a shrub that is widely distributed in tropical Africa and has been used in traditional medicine for various purposes, including the treatment of fever, rheumatism, and gastrointestinal disorders (Akah and Okafor, 1992). The plant has been reported to possess various biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties (Kumar et al., 2013).

The antioxidant properties of *Vernonia amygdalina* are attributed to the presence of various phytochemicals, including flavonoids, phenolic acids, and terpenoids (Kumar et al., 2013). Flavonoids, in particular, have been shown to possess potent antioxidant activity, and have been implicated in the prevention of various diseases, including cancer and cardiovascular disease (Middleton et al., 2000).

Despite the reported antioxidant properties of *Vernonia amygdalina*, there is a need for further studies to fully evaluate its potential as a source of natural antioxidants. This study aims to evaluate the antioxidant properties of *Vernonia amygdalina* and to identify the phytochemicals responsible for its antioxidant activity. The leaves of *Vernonia amygdalina* are commonly used in traditional medicine, and have been reported to possess various biological activities. However, the antioxidant properties of the leaves have not been fully evaluated. This study will investigate the antioxidant activity of the leaves of *Vernonia amygdalina* using various in vitro assays.

The results of this study will contribute to the conservation and sustainable use of *Vernonia amygdalina*, which is an important plant species in African traditional medicine. The study will also provide valuable information on the antioxidant properties of *Vernonia amygdalina*, which can be used to develop new natural antioxidants for the prevention and treatment of various diseases.

MATERIALS AND METHODS

Sample Collection

The medicinal plant used in this study was the Bitterleaf. They were obtained from Eke Oko market, Anambra state Nigeria and identify at Botany dept of NAU. The samples were immediately transported to the laboratory for use.

Sample Preparation

The samples were shredded into very small sizes and sun dried for 7 days to constant weight. This was other ground into fine powder and stored in an air tight plastic container for extraction.

Extraction of Plant

Cold maceration using Methanol

500g of powdered sample was weighed into a glass and 500ml extracting solvent (Methanol) was added until the medicinal plant residues were fully immersed. The vessel was closed with a tight-fitting glass cover and the contents in the vessel were shaken and left to stand for three days (72 h) but with subsequent agitation until this period was over. The contents of the flask were then strained through clean pieces of cotton cloth placed on top of filter paper, both supported by a funnel, and the extracted solution (miscella) collected in a flask with a tight-fitting cover. The volume of the yield was noted. The methanoic extract was transferred into a desiccator for further drying.

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Evaluation of Anti-Oxidant Potentials of *V. amygdalina*

The antioxidant potentials of bitter leaf was evaluated using DPPH acidic and scavenging activity and FRAP assays

Evaluation of Antioxidant Potential of *V. amygdalina* using DPOPH Radical Scavenging Activity Assay

The ability of *V. amygdalina* leaves extracts to scavenge stable DPPH radical was measured using the method of (Rumengan *et al.,* 2019). Five different concentrates ranged from 10 – 50 µg/mlin methanol were prepared in test tubes. One milliliter of 0.3 mM of freshly prepared DPPH solution in methanol was added to 2.5 ml solution in each tube and allowed to react in the dark at room temperature for 30 min. Absorbance of each solution was measured at 518 nm. As a blank, 1 ml of methanol was added to 2.5 ml of each extract solution with no DPPH. As negative control, 1 mL of 0.3 mM DPPH solution added to 2.5 mL of methanol. Percentage DPPH inhibition activities of the extracts and standards were determined using the equation

$$\text{Scavenging activity (\%)} = [(Abs_{control} - Abs_{sample}) / (Abs_{control})] \times 100$$

Where $Abs_{control}$ is the absorbance of DPPH radical + methanol, and Abs_{sample} is the absorbance of DPPH radical + sample extract/standard. The results were expressed as IC50, which means the concentration at which DPPH radical was quenched by 50%. The concentration of extract providing 50% inhibition (IC50) was calculated from the plotted graph of % inhibition versus concentration curve.

Evaluation of Antioxidant Potential of *V. amygdalina* using Ferric-Reducing Antioxidant Power (FRAP)

The reducing power of the ethanol extract was determined by the method of Hseu et al. [2017]. Five different concentrates ranged from 10 – 50 µg/mlin methanol were prepared in test tubes and ascorbic acid were mixed individually to the mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide $[K_3Fe(CN)_6]$ (1% w/v). The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid (1% w/v). The samples were then centrifuged at 4000 rpm for 15 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.5% w/v). The absorbance was measured at 700 nm against a blank sample. Incubation with water in place of additives was used as blank while l-ascorbic acid was used as positive control. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract. The reducing power (%) was calculated based on the following formula:

$$\text{Reducing power (\%)} = [(A_T - A_B)/A_T] \times 100$$

Where A_B and A_T are the absorbance of blank and plant material respectively. Effective concentration, EC_{50} value of each extract was estimated from the plotted graph of percentage reducing power versus concentration of extract.

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RESULT

DPPH Radical Scavenging Activity

DPPH radical scavenging values of bitter leaf extract are presented in Table 1. It exhibited appreciable scavenging activity ranging from 81.84% to 88.95%.

Table 1: Antioxidant Activity of Bitter Leaf Extract by DPPH Radical Scavenging Activities

Concentration Of Sample Used (µg/ml)	Absorbance Of Sample			Mean Absorbance	% Inhibition
	1ST RUN	2ND RUN	3RD RUN		
10	0.160	0.161	0.163	0.161	81.84±0.500
20	0.153	0.152	0.156	0.154	82.63±0.307
30	0.124	0.122	0.125	0.124	86.02±0.437
40	0.106	0.104	0.101	0.104	88.27±0.330
50	0.095	0.100	0.098	0.098	88.95±0.304

Each value is expressed as mean ± SD (n =3)

The results were expressed as IC50, which means the concentration at which DPPH radical was quenched by 50%. The concentration of extract providing 50% inhibition (IC50) was calculated from the plotted graph of % inhibition versus concentration curve shown in fig 1 below

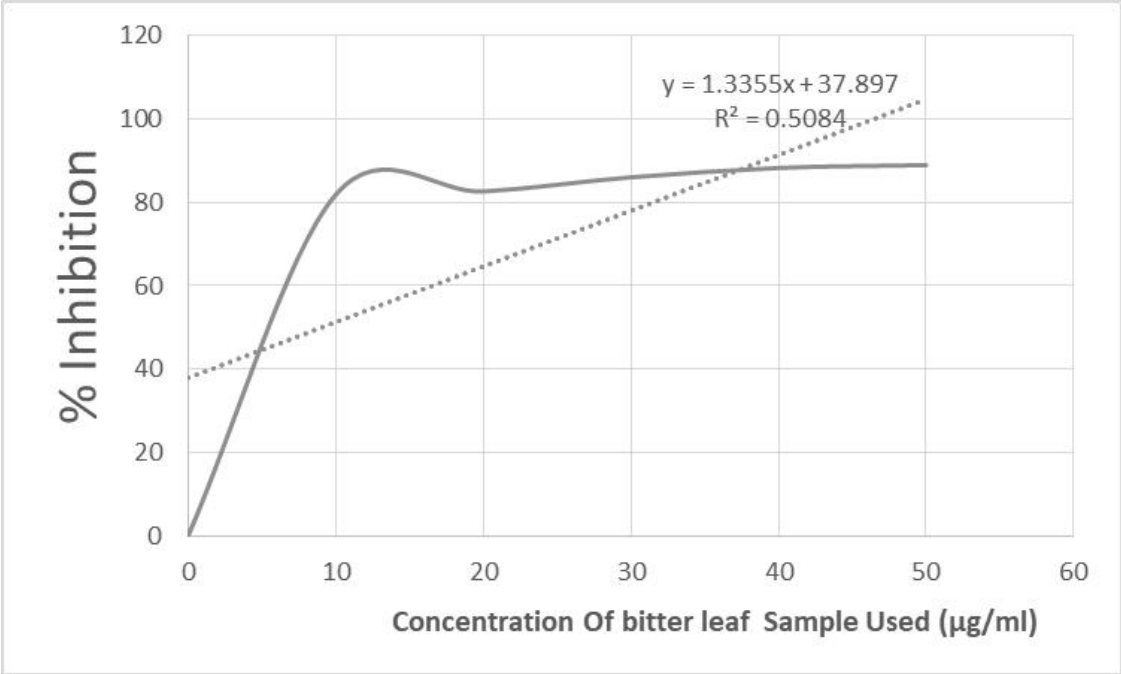

Fig. 1: Graph of % Inhibition versus Concentration Curve

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Based on Figure 1, regression equation of the samples were obtained to determined their IC50 value as shown in Table 2.

Table 2: IC50 values of Methanolic Leaf Extracts of Bitter leaf

S/n	Regression Equation	R2	IC50 (µg/ml)
1	y = 1.3355xx + 37.89	0.5084	8.13

Ferric-Reducing Antioxidant Power (FRAP)

Results of reducing power of bitter leaf extract are presented in Table 3. It exhibited appreciable scavenging activity ranging from 81.50% to 88.91% .

Table 3: Antioxidant Activity of Bitter Leaf Extract by Ferric-Reducing Antioxidant Power (FRAP)

Concentration Of Sample Used (µg/ml)	Absorbance Of Sample			Mean Absorbance	Amount of Fe (II) liberated µg/ml)
	1ST RUN	2ND RUN	3RD RUN		
10	0.044	0.045	0.045	0.045	0.608±0.301
20	0.071	0.073	0.075	0.073	1.042±0.430
30	0.10	0.11	0.11	0.11	1.650±0.110
40	0.152	0.155	0.150	0.152	2.86±0.350
50	0.15	0.18	0.20	0.18	2.43±0.300

Each value is expressed as mean ± SD (n =3)

The results were expressed as EC50, which means the concentration at which Fe (II) liberated µg/ml) by 50%. The concentration of extract providing 50% inhibition (EC50) was calculated from the plotted graph of % inhibition versus concentration curve shown in fig 2 below.

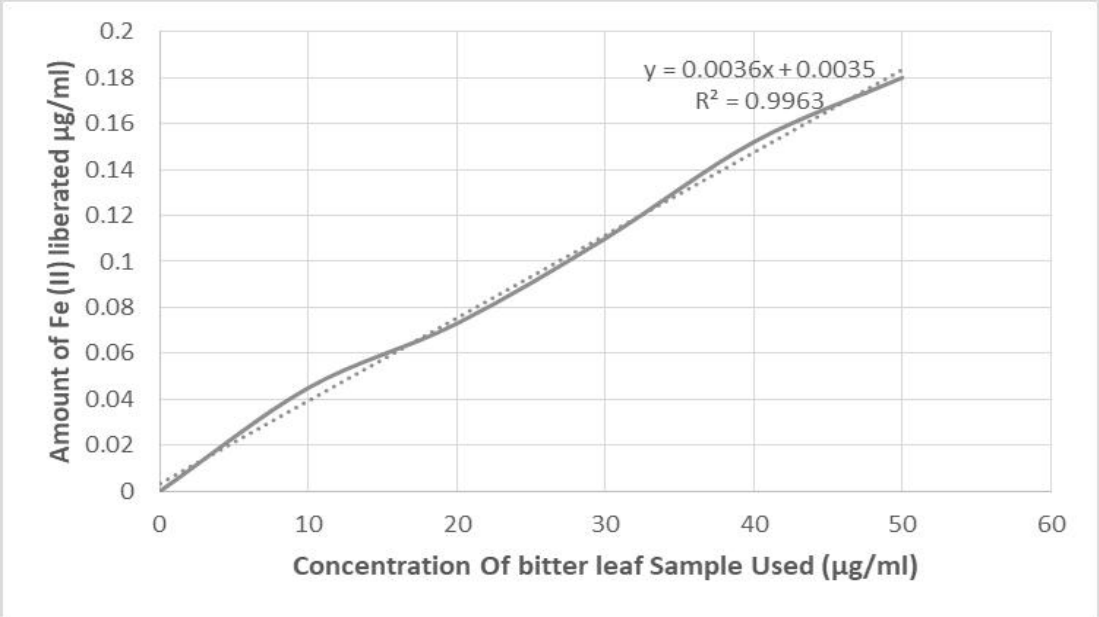

Fig. 2: Graph of % Inhibition versus Concentration Curve for 50% inhibition (EC50)

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Based on Figure 2, regression equation of the samples were obtained to determined their EC50 value as shown in Table 4.

Table 4: Effective Concentration (EC50) values of Methanolic Leaf Extracts of Bitter Leaf

S/n	Regression Equation	R2	EC50 (µg/ml)
1	y = 0.0036+ 0.0035	0.9963	24.31

Discussion

The antioxidant potentials of *Vernonia amygdalina* (bitter leaf) were evaluated using two standard methods—DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and Ferric-Reducing Antioxidant Power (FRAP). The DPPH assay recorded high free radical scavenging activity across all concentrations (10–50 µg/ml), with percentage inhibition ranging from 81.84% to 88.95%. At 10 µg/ml, the extract recorded a percentage inhibition of 81.84%, increasing progressively to 88.95% at 50 µg/ml. These values suggest that bitter leaf possesses strong antioxidant activity in a dose-dependent manner.

When compared to the findings of Oyedemi *et al*. (2020), who reported 74.5% DPPH inhibition for methanolic extracts of *Vernonia amygdalina* at 100 µg/ml, the current study demonstrates a higher antioxidant capacity even at lower concentrations, which may be attributed to better extraction efficiency, fresher plant material, or regional phytochemical variation. The study shows that *V. amygdalina posses an IC50 value of* 18.13 µg/ml. This is significantly lower to an IC50 value of 18.70 µg/ml reported by Adesanoye and Farombi (2014). The IC50 value gotten from this study as compared to 18.70 µg/ml gotten by Adesanoye and Farombi (2014) indicates a stronger scavenging ability, as a lower concentration is needed to scavenge 50% of free radicals. This variation may arise from differences in solvent polarity, plant age, geographical origin, or post-harvest processing conditions**.**

Furthermore, the results are consistent with the findings of Oboh *et al.* (2021), who demonstrated high antioxidant activity in green leafy vegetables, including *V. amygdalina*, with DPPH inhibition values of over 80%. The similarity affirms the role of polyphenols and flavonoids present in the extract, which are potent hydrogen donors and electron carriers. However, the slight increase in values in the current study could be linked to the higher concentration of these phytochemicals due to methanolic extraction, which is known for its efficacy in solubilizing antioxidant compounds (Ijeh *et al.,* 2024).

The importance of this high DPPH radical scavenging activity lies in its potential to neutralize free radicals in the human body, thereby reducing oxidative stress, which has been implicated in aging, inflammation, and chronic diseases such as cancer and cardiovascular disorders.

In the FRAP assay, the reducing power of *Vernonia amygdalina* also showed a dose-dependent trend. The amount of Fe(II) liberated ranged from 0.608 µg/ml at 10 µg/ml extract concentration to 2.86 µg/ml at 40 µg/ml. Interestingly, at 50 µg/ml, the reducing power slightly decreased to 2.43 µg/ml. This slight drop may be due to the saturation or antagonistic interaction of phytoconstituents at higher concentrations.

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The EC50 value for the FRAP assay was found to be 24.31 µg/ml, which is relatively lower than the value reported by Ezekiel *et al.* (2017), who obtained an EC50 of 36.25 µg/ml for the aqueous extract of *V. amygdalina*. This supports the observation that methanol is more efficient than water in extracting phenolic compounds, resulting in higher reducing power. Similarly, Akinmoladun *et al*. (2020) reported lower reducing power of *V. amygdalina* in their study due to the crude nature of the extract, lacking purification steps that concentrate antioxidant constituents.

Moreover, the results of the FRAP assay confirm the antioxidant properties of *V. amygdalina*, reflecting its electron-donating ability, which is crucial in breaking the chain of oxidative reactions and maintaining cellular integrity.

The DPPH test showed the highest activity at 88.95% with an IC50 of 8.13 µg/ml, whereas the FRAP assay peaked at 2.86 µg/ml with an EC50 of 24.31 µg/ml. Thus, DPPH assay results indicate a stronger antioxidant potential. The difference may be attributed to the nature of the assays—DPPH measures hydrogen atom transfer while FRAP measures electron transfer, and various compounds in the extract may act preferentially in one mechanism over the other (Prior *et al.,* 2015).

Conclusion

The study confirmed that *Vernonia amygdalina* (bitter leaf) has strong antioxidant properties, demonstrated by its high DPPH scavenging activity and ferric-reducing power. The low IC_{50} (8.13 µg/ml) and EC_{50} (24.31 µg/ml) values indicate its potency in neutralizing free radicals. These findings support its traditional use and highlight its potential in developing natural antioxidant therapies for managing oxidative stress-related diseases.

Recommendations

Based on the findings of the study, the following recommendations were made:

1. **Further phytochemical analysis** should be conducted to isolate, characterize, and identify the specific bioactive compounds responsible for the antioxidant properties of *Vernonia amygdalina*.
2. **In vivo studies** are recommended to evaluate the physiological and therapeutic relevance of the antioxidant properties of the extract in animal models or clinical trials.
3. **Comparative studies using different solvents and extraction methods** should be carried out to optimize the extraction of antioxidant compounds from *Vernonia amygdalina*.
4. **Standardization and formulation of dietary supplements or pharmaceutical products** based on *Vernonia amygdalina* should be encouraged to harness its therapeutic potential.
5. **Public awareness programs** should be initiated to promote the inclusion of *Vernonia amygdalina* in daily diets, especially in communities where oxidative stress-related diseases are prevalent.

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