# Phytochemical and Antioxidant Analysis of the Chloroform Extract of Bitter Leaf (*Vernonia amygdalina*) Flower

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***Abstract***: *The phytochemical composition and antioxidant activity of the chloroform extract (CBL) of the flower of Vernonia amygdalina were investigated. The flower samples were collected, air-dried, and subjected to sequential extraction using n-hexane followed by chloroform. The chloroform extract was obtained with a percentage yield of 0.576%. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, and glycosides, while tannins and saponins were absent. The chemical profile of the extract was further evaluated using thin-layer chromatography (TLC), which revealed 5 to 8 distinct phytochemical spots depending on the solvent system employed. The best resolution was obtained using chloroform:n-hexane (3:1), yielding eight spots with Rf values ranging from 0.01 to 0.90, indicating the presence of compounds with varying polarities. The antioxidant activity of CBL was assessed using the DPPH radical scavenging assay. The extract exhibited a concentration-dependent increase in antioxidant activity, with the highest inhibition observed at the highest concentration tested (0.4 mg/mL), while the lowest activity was recorded at the most diluted concentration (0.025 mg/mL). This confirms a strong dose-dependent free radical scavenging potential. Overall, the results demonstrate that the chloroform extract of Vernonia amygdalina flower contains diverse bioactive phytochemicals and exhibits significant antioxidant activity, supporting its potential application in pharmaceutical and nutraceutical development.*

***Keywords:*** *Vernonia amygdalina, chloroform extract, phytochemical screening, thin-layer chromatography (TLC), antioxidant activity, DPPH assay*

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## 1.0 Introduction

Bioactive molecules that occur in nature have served as a reservoir of medicinal substances, contributing significantly to pharmacological discovery (Newman & Cragg, 2020). Structurally diverse natural products such as: alkaloids, saponins, flavonoids, terpenoids, and glycosides, are synthesized by plants and are known bioactive compounds of significant relevance in medicinal chemistry. They exhibit activities such as antioxidant, antimicrobial, anti-inflammatory and anticancer (Croteau *et al*., 2000; Wink, 2015). These bioactive compounds play a crucial role in the discovery and development of novel therapeutic agents. One of the major mechanisms through which these phytochemicals exert their therapeutic effects is through antioxidant activity. Free radicals such as hydroxyl and superoxide anions have been implicated in oxidative stress induced pathologies (Halliwell & Gutteridge, 2015). Free radicals scavengers of plant origins have been reported in literature to have mitigated oxidative damage (Prior *et al*., 2005).

*Vernonia amygdalina a member of the Asteraceae family, is* commonly known as bitter leaf, and is widely distributed across tropical regions of Africa. .It is a nutraceutical plant with well-documented ethnomedicinal

applications , including the treatment of malaria, gastrointestinal disorders and metabolic-related diseases (Izevbigie, 2003; Farombi & Owoeye, 2011). The leaves of *V. amygdalina* have been studied extensively. While the leaves of *V. amygdalina* have been extensively studied, there is limited information on the phytochemical composition, chromatographic profile, and antioxidant potential of its floral parts, particularly when extracted using solvents of intermediate polarity such as chloroform. Recent studies have reported the phytochemical and antioxidant properties of crude ethanolic extracts of *V. amygdalina* flowers (Odokwo & Eze, 2024); however, detailed studies on solvent-partitioned extracts remain scarce (Odokwo & Eze, 2024), reflecting a relative scarcity of data on its phytochemical composition and bioactivity. This study aims to characterize the phytochemical constituents, evaluate the chromatographic profile using thin-layer chromatography (TLC), and assess the antioxidant potential of the chloroform extract of *Vernonia amygdalina* flower.

"The findings of this study will provide valuable insights into the bioactive potential of the floral part of the plant and may support its application in the development of natural antioxidant agents for pharmaceutical and nutraceutical purposes."

## 2.0 Materials and Methods

### *2.1 Equipment and Apparatus*

The equipment used in this study included an analytical electronic weighing balance (Mettler JA 3003A), rotary evaporator (RE100-S), 500 mL beakers, 250 mL measuring cylinders, pre-coated thin-layer chromatography (TLC) plates, separating funnel, retort stand, and capillary tubes.

### *2.2 Reagents and Chemicals*

All reagents used were of analytical grade and were used without further purification. The chemicals employed in this study included chloroform, n-hexane, Hager's reagent (saturated picric acid solution), 10% lead acetate solution, ferric chloride ($FeCl_3$), concentrated sulfuric acid ($H_2SO_4$), bromine water, distilled water, iodine granules, and methanol.

### *2.3 Collection and Preparation of Plant Material*

Fresh flowers of Vernonia amygdalina (bitter leaf) were collected from Atai Obio Offor community in Uyo Local Government Area of Akwa Ibom State, Nigeria. The plant material was authenticated at the Department of Biology, Federal University Otuoke, Nigeria.

The collected flowers were washed to remove debris, air-dried at ambient temperature, and pulverized into a fine powder using a mechanical grinder. The powdered sample was stored in an airtight container prior to extraction.

### *2.4 Extraction of Phytochemicals*

A total of 33.1131 g of the powdered flower sample was weighed and transferred into a 500 mL beaker. The sample was first macerated with 100 mL of n-hexane for 72 hours at room temperature with intermittent stirring. The mixture was then filtered, and the residue was subsequently re-extracted with chloroform for another 72 hours.

The chloroform extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain the crude chloroform extract (CBL). The percentage yield of the extract was calculated using equation (1)

$$\%yeild = \frac{Weight\ of\ extract}{Weight\ of\ plant} \times \frac{100}{1} \qquad (2)$$

### *2.5 Qualitative Phytochemical Screening*

Qualitative phytochemical analysis of the chloroform extract (CBL) was carried out using standard procedures as described by Odokwo & Eze (2024).

#### *2.5.1 Test for Alkaloids*

To 2 mL of CBL, a few drops of Hager's reagent were added. The formation of a yellow precipitate indicated the presence of alkaloids.

***2.5.2 Test for Flavonoids***

To 1 mL of CBL, a few drops of 10% lead acetate solution were added. The appearance of a yellow coloration indicated the presence of flavonoids.

***2.5.3 Test for Steroids (Salkowski Test)***

To 5 mL of CBL, 2 mL of chloroform and 3 mL of concentrated $H_2SO_4$ were carefully added. The formation of a reddish-brown ring at the interface indicated the presence of steroids (terpenoids).

***2.5.4 Test for Tannins***

To the extract, 10 mL of bromine water was added. Decolorization of the bromine water indicated the presence of tannins.

***2.5.5 Test for Saponins***

Approximately 200 mg of CBL was mixed with 5 mL of distilled water and shaken vigorously. A few drops of olive oil were added and shaken further. Persistent foam formation indicated the presence of saponins.

***2.5.6 Test for Glycosides***

To 1 mL of CBL, distilled water and a few drops of Molisch reagent were added, followed by careful addition of concentrated $H_2SO_4$ along the side of the test tube. The formation of a violet ring indicated the presence of glycosides.

***2.6 Thin Layer Chromatography (TLC) Analysis***

Thin-layer chromatography (TLC) was carried out to determine the number of phytochemical constituents present in the chloroform extract. Pre-coated TLC plates were used, and the extract was applied using capillary tubes.

The plates were developed in solvent systems consisting of n-hexane and chloroform in the ratios of 0:3, 1:3, and 2:3. After development, the plates were visualized, and the retention factor (Rf) values were calculated for the separated components.

***2.7 Antioxidant Activity (DPPH Assay)***

The antioxidant activity of the chloroform extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method.

A stock solution of 0.4 mg/mL of CBL was prepared in methanol. Serial dilutions were carried out to obtain concentrations of 0.2, 0.1, 0.05, and 0.025 mg/mL. All analyses were performed in triplicate, and the mean values were recorded. Ascorbic acid was used as the standard antioxidant.

To each test solution, 2 mL of DPPH solution was added and incubated in the dark for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. A control containing DPPH solution without extract was used as the blank.

The percentage inhibition (%I) of DPPH radicals was calculated using equation 2

$$\%I = \frac{A_{BLANK} - A_{CBL}}{A_{BLANK}} \quad (2)$$

where: $\%I$ – percentage inhibition, $A_{BLANK}$ – Blank absorbance, and $A_{CBL}$ –chloroform solution absorbance

***2.8. Statistical Analysis***

Data obtained were expressed as mean of triplicates determinations ± standard deviation (SD). The Statistical Package for Social Scientists (SPSS version 20.0) was used for all data analysis.

**3.0 Results and Discussion**

***3.1. Extraction and Yield***

The chloroform extract of *Vernonia amygdalina* flower (CBL) was obtained as a greenish residue, indicating the effective solubilization of chlorophyll-associated compounds and other moderately polar phytoconstituents into the chloroform solvent system. As presented in Table 1, the percentage yield of the extract was 0.576%, corresponding to 0.191 g of extract obtained from 33.1131 g of dried plant material. This relatively low yield suggests that chloroform selectively extracted a fraction of the total phytochemical content, particularly compounds of intermediate polarity, while excluding highly

polar constituents such as carbohydrates and proteins, as well as non-polar compounds that were likely removed during the initial n-hexane extraction stage.

**Table 1: Yield of CBL**

| Initial mass of materials (g) | Mass of CBL (g) | %Yield |
|---|---|---|
| 33.1131 | 0.191 | 0.576 |

The extraction efficiency observed in this study is consistent with solvent-partitioning approaches, where the goal is not maximal yield but selective enrichment of specific phytochemical classes. Similar yield trends have been reported for chloroform fractions of medicinal plants, which typically produce lower yields compared to crude methanolic or ethanolic extracts (Odokwo & Eze, 2024). Importantly, the relatively low yield does not imply low bioactivity; rather, it may indicate a higher concentration of pharmacologically active constituents within the extract.

### *3.2 Phytochemical Profiling*
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The qualitative phytochemical screening of the chloroform extract revealed the presence of alkaloids, flavonoids, steroids, and glycosides, while tannins and saponins were not detected. These findings are summarized in **Table 2** and are in agreement with previous reports on *Vernonia amygdalina* (Odokwo & Eze, 2024), although earlier studies have predominantly focused on the leaf rather than the floral component.

The presence of alkaloids in the extract is significant due to their well-documented pharmacological activities, including antimicrobial, antimalarial, and anticancer effects, which are often linked to their ability to interfere with nucleic acid synthesis and enzyme function (Stermitz et al., 2000; Cragg & Newman, 2005). Flavonoids, which were also detected, are widely recognized as potent antioxidants capable of scavenging reactive oxygen species, chelating metal ions, and modulating cellular signaling pathways (Panche et al., 2016). The detection of steroids further suggests potential anti-inflammatory and immunomodulatory properties, as steroidal compounds are known to play critical roles in regulating physiological and metabolic processes (Cidlowski, 2004; Barnes, 2011). Glycosides, which were also present, contribute to diverse biological activities, including cardioprotective and antimicrobial effects.

The absence of tannins and saponins, as indicated in Table 2, may be attributed to their higher polarity, which limits their solubility in chloroform. This observation underscores the importance of solvent selection in phytochemical extraction, as different solvents preferentially extract different classes of compounds. Overall, the phytochemical profile suggests that the flower of *Vernonia amygdalina* is a valuable source of bioactive compounds with potential therapeutic applications.

**Table 2: Qualitative Phytochemical Analysis of CBL**

| S/Nos. | Phytochemicals | Observation |
|---|---|---|
| **1.** | Alkaloids | + |
| **2.** | Flavonoids | + |
| **3.** | Steriods | + |
| **4.** | Tannins | - |
| **5.** | Saponins | - |
| **6.** | Glycosides | + |

***+: present, -: absent**

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### *3.3 Thin Layer Chromatography (TLC) Profiling*

Further insight into the chemical composition of the chloroform extract was obtained through thin-layer chromatography (TLC) analysis. As shown in Table 3, the number of resolved spots varied with the solvent system used, ranging from five spots in pure chloroform to eight spots in the chloroform:n-hexane (3:1) system. The increased number of spots observed with the mixed solvent system indicates improved separation efficiency, suggesting that the polarity of the mobile phase plays a critical role in resolving the phytochemical constituents of the extract.

The solvent system chloroform:n-hexane (3:1) provided the best resolution, yielding eight distinct spots, which implies that this ratio offers an optimal balance between polarity and elution strength. The retention factor (Rf) values obtained ranged from 0.01 to 0.90, as detailed in Table 3, indicating the presence of compounds with a wide range of polarities. Compounds with low Rf values exhibited strong interactions with the stationary phase, suggesting higher polarity, whereas those with high Rf values were less polar and more mobile in the solvent system.

The broad distribution of Rf values confirms that the extract is composed of structurally diverse phytochemicals, corroborating the results of the qualitative screening. The ability of the mixed solvent system to resolve more components highlights the importance of optimizing solvent polarity in chromatographic analysis. Furthermore, the TLC profile provides a useful chemical fingerprint for the extract, which can be employed in quality control, authentication, and comparative phytochemical studies.

### *3.4 Antioxidant Profiling*

The antioxidant activity of the chloroform extract was evaluated using the DPPH radical scavenging assay, and the results are presented in Fig. 1. The extract exhibited a clear concentration-dependent increase in percentage inhibition, with higher concentrations demonstrating greater free radical scavenging activity. Conversely, a decrease in activity was observed with dilution, confirming a well-defined dose–response relationship.

The trend observed in Fig. 1 indicates that the antioxidant capacity of the extract is directly related to the concentration of bioactive compounds capable of donating electrons or

hydrogen atoms to neutralize DPPH radicals. This behavior is characteristic of plant-derived antioxidants and suggests that the extract contains compounds with strong radical scavenging potential.

**Table 3: $R_f$ Values of the TLC Analysis of CBL**

| S/Nos. | CBL | | 6Solvent System |
|---|---|---|---|
| | No. of Spots | $R_f$ | |
| **1.** | 5 | 0.09, 0.14, 0.31, 0.53, 0.68 | 100% chloroform |
| **2.** | 8 | 0.06, 0.16, 0.23, 0.37, 0.48, 0.65, 0.79, 0.90 | Chloroform: n-hexane (3:1) |
| **3.** | 5 | 0.90, 0.73, 0.58 0.01, 0.03 | Chloroform: n-hexane (3:2) |

The antioxidant activity observed can be largely attributed to the presence of flavonoids, which are known to possess multiple hydroxyl groups that facilitate electron donation and stabilization of free radicals. Additionally, alkaloids and glycosides may contribute to the overall antioxidant effect through complementary mechanisms. The results obtained in this study are consistent with previous findings on *Vernonia amygdalina*, although most prior studies have focused on leaf extracts rather than the floral component.

From a mechanistic perspective, the antioxidant activity may involve both hydrogen atom transfer and single electron transfer processes, which are typical pathways in DPPH-based assays. The significant radical scavenging activity demonstrated by the extract suggests its potential application as a natural antioxidant in pharmaceutical and nutraceutical formulations.

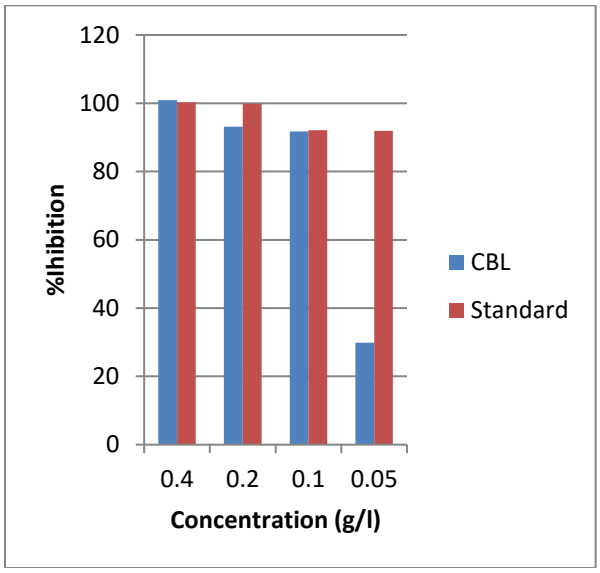


**Fig. 1: Antioxidant Activity of CBL**

The results obtained in this study collectively demonstrate that the chloroform extract of *Vernonia amygdalina* flower is rich in bioactive phytochemicals and exhibits significant antioxidant activity. The data presented in Tables 1–3 and Fig. 1 provide strong evidence of the chemical diversity and functional potential of the extract. The study not only confirms the medicinal relevance of the plant but also highlights the underexplored potential of its floral component. These findings provide a scientific basis for further investigation into the isolation, characterization, and application of the bioactive compounds present in the extract.

## 4.0 Conclusion

This study has demonstrated that the chloroform extract of *Vernonia amygdalina* flowers is a valuable source of bioactive natural products with notable antioxidant activity. Qualitative phytochemical screening revealed

the presence of key secondary metabolites, including alkaloids, flavonoids, steroids, and glycosides, which are known to contribute to various pharmacological effects.

Thin-layer chromatography (TLC) profiling further confirmed the presence of a chemically diverse mixture of phytoconstituents, indicating the complexity of the extract. In addition, the antioxidant potential of the extract, as evaluated by the DPPH assay, showed a clear concentration-dependent radical scavenging activity, highlighting its redox capability.

Overall, these findings provide scientific evidence supporting the pharmacological relevance of the floral part of *Vernonia amygdalina*, an aspect that has been relatively underexplored compared to other parts of the plant. The study therefore contributes to expanding the phytochemical and bioactivity database of this important medicinal plant.

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**Declaration**

**Consent for publication**

Not Applicable

**Availability of data and materials**

The publisher has the right to make the data public

**Conflict of Interest**

The authors declared no conflict of interest

**Ethical Considerations**

Not applicable

**Competing interest**

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**Authors' Contributions**

Edet O. Odokwo conceived and designed the study, supervised the research, performed data analysis, and prepared the manuscript draft. Martha S. Onifade contributed to sample collection, laboratory experiments, data interpretation, and manuscript review and editing. Both authors read, revised, and approved the final version of the manuscript.