

Antioxidant Activity and Bioactive Compounds of *Vernonia Amygdalina* L. Leaves in Methanolic and Aqueous Extracts

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ABSTRACT

In recognition of its numerous medicinal benefits, bitter leaf (*Vernonia amygdalina* L.) is widely used in traditional medicine. Despite substantial research, a comprehensive comparative analysis of the antioxidants and phytochemical properties of its methanolic and aqueous extracts remains undocumented. This study addresses this gap by examining the differences in antioxidant values and phytochemical constituents between the two extracts using advanced analytical techniques. NMR and FTIR spectroscopy were employed to investigate probable phytochemicals, while HPLC and GC-MS determined the differences in biological components. Both DPPH and ABTS radical-scavenging tests evaluated variations in antioxidant activity. NMR Results indicated the presence of aromatic protons, phenolic hydroxyl groups, methyl groups, and methoxylated substances by the singlet signal at 7.1–7.5 ppm, singlet at 4.0–4.5 ppm, doublet at 1.0–1.5 ppm, and singlet at 2.0–2.5 ppm, respectively. FTIR results showed a C=O stretching at 1700 cm^{-1} , an O-H stretching at about 3250 cm^{-1} , and a C=C stretching between 1500 and 1600 cm^{-1} . Methanolic extracts had higher phytochemical values according to GC-MS and HPLC data, except for rutin, delta-cadinene, and alpha-cadinol, which were higher in the aqueous extract. The methanolic extract exhibited superior antioxidant activity in DPPH and ABTS assays, with maximal scavenging activities of 90.5% and 96.8%, respectively. At 200 $\mu\text{g/mL}$, the aqueous extract demonstrated scavenging activities of 85.4% and 92.7%. These findings underscore the enhanced potential of methanolic extract for therapeutic applications.

Keywords: Antioxidant Activity, Aqueous Extracts, Methanolic Extracts, Phytochemicals, *Vernonia amygdalina* L.

1.0 Introduction

A well-known medicinal plant in many traditional medical systems, bitter leaf (*Vernonia amygdalina* L.), is especially used in African and Asian traditional medicine. Due to its many medicinal uses, this plant has been shown to have antibacterial, anticancer, antidiabetic, and anti-inflammatory qualities (Ogidi et al., 2019). Typically ranging 10–15 cm in length and 4–5 cm in breadth, the oblong-lanceolate leaves of *Vernonia amygdalina* L. are medium to dark green. To maximize the therapeutic potential of medicinal plants, it is essential to comprehend how their phytochemical content and antioxidant activity vary depending on the solvent used during extraction (Wenli Sun et al., 2023). Due to their strong antioxidant capabilities, phenolics and flavonoids (figure 1) are the main bioactive chemicals that are responsible for these health advantages (Ma'aruf et al., 2024 & Egharevba et al., 2020).

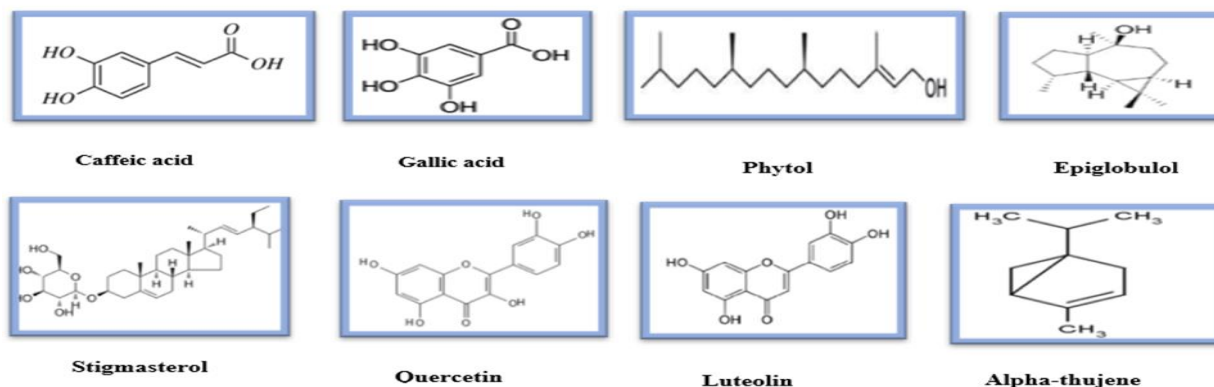


Figure 1: Structure of some phenolics and flavonoids

Plants possess phytochemicals including flavonoids, terpenoids, and phenolic acids, which are essential for their medicinal properties. Strong antioxidant properties of phenolic substances, such as gallic acid, chlorogenic acid, and caffeic acid, aid in scavenging free radicals and lowering oxidative stress (Kang et al., 2023). The plant's anti-inflammatory and anticancer effects are attributed to flavonoids such as kaempferol, quercetin, and rutin (Martha et al., 2023). The solvent used to extract these bioactive substances can have a big impact on how effective they are (Arawande et al., 2022).

The phytochemical, proximate, and mineral contents of the leaves, stems, and roots of the *Vernonia amygdalina* L. (bitter leaf) plant were evaluated using the standard method of the Association of Analytical Chemists (Ma'aruf et al., 2024) and Atomic Absorption Spectrophotometric (Ogidi et al., (2019). Numerous phytochemicals were discovered to be present in the plant sample. Alkaloids were measured quantitatively, and the results showed that tannins were at $6.30 \pm 3.44\%$, saponins were at $3.20 \pm 1.40\%$, flavonoids were at $2.37 \pm 0.68\%$, terpenoids were at $1.30 \pm 1.18\%$, and phenols were at $0.54 \pm 0.36\%$.

Muhammed et al. (2020) used the nutrient agar diffusion method to analyze *Vernonia amygdalina* L. for phytochemicals and antimicrobials. The phytochemical screening revealed the presence of bioactive components. The antibacterial activity against *Staphylococcus aureus* displayed the largest zones of inhibition in (7.5 mm). Rumengan et al. (2020) divided nanochitosan coated extracts into two groups, corresponding to a 1:1 (BLENC 1) and a 2:1 (BLENC 2) ratio of nanochitosan to extract. Using the DPPH test for the parameter of inhibitory values, the antioxidant activity of the two groups with varying concentrations (10 to 50 µg/ml) and the control (BLE) was determined. Their findings indicate that at 50 µg/ml, BLENC 2 exhibits a greater inhibition value (89.1%) in comparison to BLENC 1 at 50 µg/ml. All samples exhibited an inhibition value within the extremely strong antioxidant category, with an IC50 ranging from 8.98 to 22.25-µg/ml. The concentration vs inhibition value graph's linear regression reveals that BLENC was the location of the correlation 2 ($R^2=0.828$). Imaga and Bamigbetan's (2013) evaluated the antioxidant content of *Vernonia amygdalina* aqueous extracts. Antioxidants such as glutathione, superoxide dismutase, catalase, and malondialdehyde were found in higher concentrations in the test rats according to in vivo experiments. The results support the use of the extract as a food supplement and herbal remedy by indicating that it strengthens the antioxidant system without being harmful.

Using a UV-Vis spectrophotometer set at 516 nm and vitamin C as a reference, Karlina et al. (2023) assessed the antioxidant activity of a pure extract from *Vernonia amygdalina* L. leaves. Drying shrinkage of $0.79 \pm 0.02\%$, water content of $7.33 \pm 2.08\%$, water-soluble essence of $20.5 \pm 1.73\%$, and ethanol content of $14.5 \pm 4.35\%$ were all observed in the leaf extract. The presence of tannins, steroids, flavonoids, and saponins was determined by chemical analysis. Similar to quercetin, flavonoids with Rf values of 0.68 and 0.5 were detected by thin-layer chromatography. With an IC50 value of 13.54 ± 0.10 µg/mL, the purified extract demonstrated very strong antioxidant activity, suggesting its potential as a potent antioxidant.

The effectiveness of bioactive compounds can be significantly influenced by the solvent employed to extract them (Arawande et al., 2022). The extracts of bitter leaves were previously studied for their phytochemical and antioxidant value (Hasibuan et al., 2023). Despite the substantial research on *Vernonia amygdalina* L., an advanced comparative study on the phytochemical and antioxidant properties of its methanolic and aqueous extracts remains undocumented. In this study, we have addressed this gap by examining the differences in antioxidant values and phytochemical constituents between the two extracts using advanced analytical techniques. Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FTIR) spectroscopy were employed initially to investigate the presence of probable phytochemicals. Subsequently, Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) were used to identify variations in the actual biological constituents between the extracts. The differences in antioxidant activity were assessed using both DPPH and ABTS radical scavenging assays.

2.0 Materials and Methods

2.1 Preparing Plant Materials and Extracts

In the Gangrar Indian Forest, fresh leaves of *Vernonia amygdalina* L., also referred to as bitter leaf, were gathered from a nearby garden. A botanist verified the authenticity of the leaves (MUH-2024-VA-068). After carefully washing the gathered leaves in distilled water to get rid of any dirt (Ma'aruf et al, 2024), they were allowed to air dry for two weeks at room temperature. After being dried, the leaves were powdered finely with an electric grinder and kept in sealed jars at room temperature.

2.2 Solvent Extraction

(a) Methanolic Extract Preparation

The (Harborne, 1998) methodology was modified and implemented. Using a Soxhlet extractor, 40 grams of the powdered leaf material was extracted for eight hours using 400 mL of methanol. After that, a rotary evaporator was used to concentrate the methanol extract under lower pressure in order to produce a crude extract.

(b) Aqueous Extract Preparation

For the aqueous extraction, a modified version of the (Parekh & Chanda, 2007) method was used. A 400 mL of distilled water was used to soak 40 grams of the powdered leaf material for one and a half hours at 75 °C. To create a crude aqueous extract, the mixture was filtered, and the filtrate was freeze-dried.

2.3 Nuclear Magnetic Resonance (NMR)

Spectroscopy

NMR spectroscopy was used to determine the principal components' structures in the methanol extract. Prior to conducting phytochemical analysis, NMR elucidation was done in the same manner as FTIR in order to estimate the possible presence of active biochemicals. Using a Bruker Avance III 400 MHz spectrometer (Usman et al., 2024), ¹H spectra were captured. After dissolving the samples in deuterated methanol, chemical shifts were expressed as parts per million (ppm) in relation to an internal standard, tetramethylsilane (TMS) (Huang et al., 2023).

2.4 Fourier Transform Infrared Spectroscopy (FTIR) Identification

FTIR analysis was performed to identify the functional groups that were present in the extracts (Sulaiman et al., 2024). Prior to doing a phytochemical examination, this was also done to estimate the possible presence of active biochemical. The spectra were recorded on a Thermo Nicolet 6700 FTIR spectrometer (Usman et al., 2024), which has a resolution of 4 cm⁻¹ and a range of 4000-400 cm⁻¹. The samples were prepared by mixing the extracts with potassium bromide (KBr) and pressing the mixture into pellets (Ordoudi et al., 2023).

2.5 Total Phenolic Content and Total Flavonoids Content (TPC & TFC)

According to Singleton, Orthofer, and Lamuela-Raventós (1999), the Folin-Ciocalteu method was utilized to ascertain the total phenolic content. The aluminum chloride colorimetric method was used to determine the total flavonoid concentration (Huang et al., 2023).

2.6 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

A GC (Agilent 7890A with a 5975C inert XL EI/CI MSD and equipped with a Triple-Axis Detector) was used to perform the GC-MS study. The split ratio was 10:1, and the injection volume was 1 µL. By comparing the mass spectra of the compounds with the NIST library, the compounds were identified (Prakash, 2023).

2.7 High-Performance Liquid Chromatography (HPLC) Analysis

An Agilent 1200 series HPLC system with a diode-array detector (DAD) was used to conduct the HPLC analysis. With the help of a C18 column (250 mm × 4.6 mm, 5 µm particle size), the chromatographic separation was accomplished. A gradient of acetonitrile (B) and water (A), both containing 0.1% formic acid, made up the mobile phase. The following was the gradient elution: For re-equilibration, the following time intervals are given: 0–5 minutes, 10% B; 5–25 minutes, 10–60% B; 25–30 minutes, 60–90% B; 30–35 minutes, 90% B; and 35–40 minutes, 10% B. The injection volume was 20 µL, and the flow rate was 1 mL/min. For the phenolic chemicals, the detection wavelengths were chosen at 280 nm and 330 nm (Tamhid et al., 2023).

2.8 Antioxidant Activity Analysis

2.8.1 DPPH Radical Scavenging Activity

Two experiments were carried out to assess the antioxidant activity of the aqueous and methanolic leaf extracts of *Vernonia amygdalina L.* The extracts' DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined using Blois's (1958) technique with minor adjustment. Using the following formula, the proportion of DPPH radical scavenging activity was determined:

$$\begin{aligned} \text{DPPH Scavating activity} \\ &= \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \end{aligned}$$

2.8.2 ABTS Radical Scavenging Activity

For the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging test, the method outlined by Re et al. (1999) was employed. The percentage of ABTS radical scavenging activity was calculated using the formula below:

$$\begin{aligned} \text{ABTS Scavating activity} \\ &= \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \end{aligned}$$

Each experiment was run in triplicate, and the findings were expressed as mean ± standard deviation (SD). The statistical analysis was done with SPSS (Statistical Package for the Social Sciences). Significant differences between means were found using one-way ANOVA, and if $p < 0.05$, Tukey's post hoc test was used to determine whether the difference was statistically significant (McHugh, 2011).

3.0 Results

3.1 Functional Group Analyses using FTIR and NMR Spectroscopy

Prior to the subsequent advanced phytochemical study, FTIR and NMR analyses were performed to assess the possible existence of active biochemical components in both extracts. While NMR spectroscopy was used to evaluate the methanolic extracts, FTIR analysis was performed on the aqueous extracts.

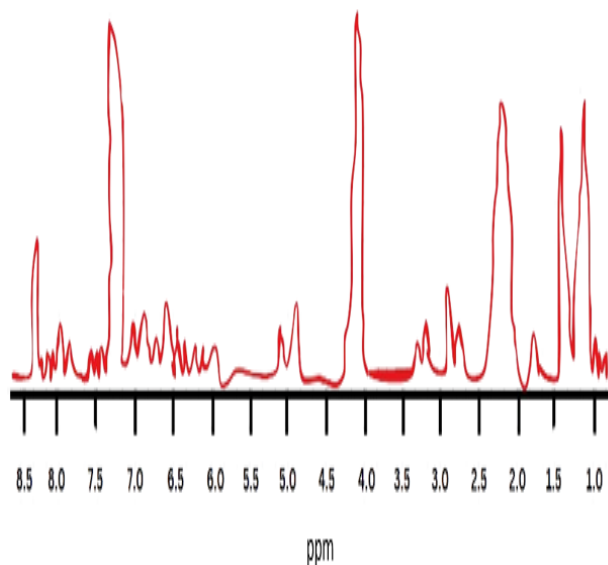


Figure 2: NMR Spectrum of Methanolic Extract of *Vernonia amygdalina L.* Leaves

Different signals detected at particular chemical shift regions (ppm) in the methanolic extract of *Vernonia amygdalina L.* leaves, as depicted in Fig. 2, indicated the probability of discovering phytochemical substances in the NMR spectrum. Aromatic protons are indicated by the singlet signal at 7.1–7.5 ppm, which implied the presence of aromatic substances (Fazlyyyakhmatov et al., 2023), including phenolics and flavonoids. The presence of alcohols or phenolic hydroxyl groups was indicated by a singlet detected at 4.0–4.5 ppm, which further supported the existence of phenolic chemicals in the extract (Lozano et al., 2023). The doublet at 1.0–1.5 ppm is indicative of methyl groups, which were frequently present in a range of secondary metabolites, such as steroids and terpenoids (Takamatsu et al., 2023). Methoxylated substances were indicated by a singlet at 2.0–2.5 ppm, which could be attributed to methoxy groups (Kostopoulou et al., 2021). All of these results

pointed to the methanolic extract's potential for phytochemicals (Zi, 2024).

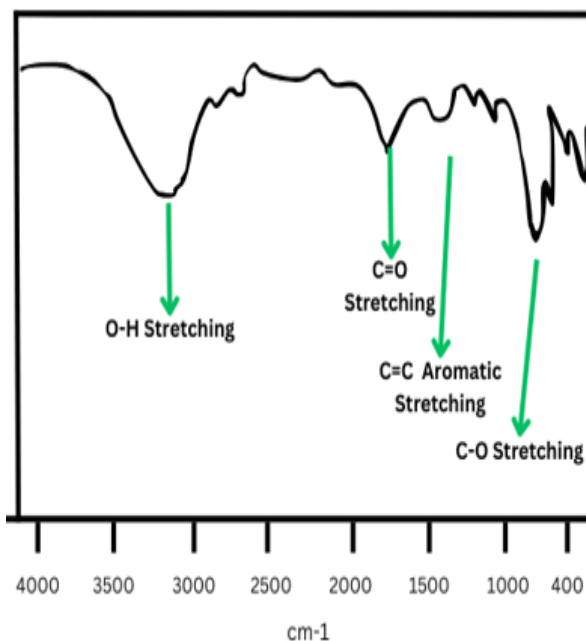


Figure 3: FTIR Pattern of Aqueous Extract of *Vernonia amygdalina L.* Leaves

The above FTIR spectra in figure 3 revealed strong absorption bands that are typical of substances containing phenol. The wide O-H stretching band at around 3250 cm^{-1} is suggestive of hydroxyl groups, which are frequently found in compounds that are phenolic (Bhattacharyya et al., 2021). About 1700 cm^{-1} , a sharp C=O stretching band indicates the presence of carbonyl groups (Sulaiman et al., 2023), which are generally present in phenolic acids and flavonoids (Batinić et al., 2024). The presence of these bioactive chemicals is further supported by the aromatic C=C stretching bands that have been detected between 1500–1600 cm^{-1} (Li et al., 2019). These results agree with FT-IR analyses of *Vernonia amygdalina L.* that have been published in the past (Sintayehu, 2016).

3.2 Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The methanolic extract had a significantly higher total phenolic content (86.7 mg/g) than the aqueous extract (71.2 mg/g) and a higher total flavonoid content (56.2 mg/g) than the aqueous extract (49.8 mg/g) as shown in figure 4. This suggests that because of its polarity, which enables it to solubilize a wider variety of phytochemicals, including those with different polarities, methanol is a more efficient solvent for extracting these beneficial substances. The higher TPC and TFC in the methanolic

extract imply that it may have a stronger antioxidant capacity and greater therapeutic potential (Gushiken et al., 2022).

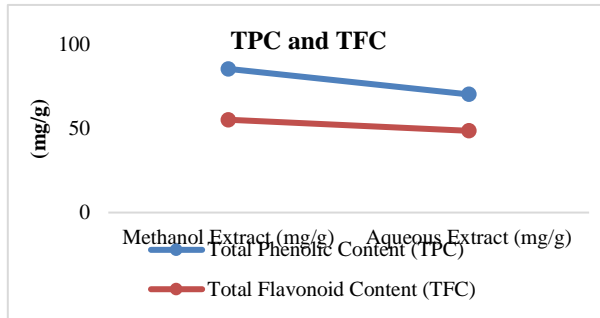


Figure 4: Variation of TPC and TFC in Methanolic and Aqueous Extracts

3.3 Determination of Phytochemical Constituents using GC-MS

GC-MS is essential for detecting phytochemical constituents (Mustapha et al., 2024). The GC-MS analysis identified several key volatile and semi-volatile compounds in the methanol and aqueous extract of *Vernonia amygdalina L.* leaves. The major compounds detected are summarized in table 1.

Table 1: Variations of Phytochemicals in Methanolic and Aqueous Extract by GC-MS

Compound	Methanolic Extract (%)	Aqueous Extract (%)
Alpha-thujene	1.4	1.5
Caryophyllene	4.6	3.4
Germacrene D	5.8	5.0
Beta-selinene	2.9	2.3
Alpha-cadinol	0.8	1.1
Delta-cadinene	0.6	0.8
Epiglobulol	2.7	1.9
Phytol	2.4	1.7
Lupeol	0.9	0.5
Stigmasterol	1.2	1.0

Different phytochemicals were detected in the methanolic and aqueous extracts of *Vernonia amygdalina L.* leaves by GC-MS analysis; however, there were significant variations in the quantities of these phytochemicals between the two extracts (table 1). Similar concentrations of alpha-thujene—1.4% in the methanolic extracts and 1.5% in the aqueous extract were detected in both preparations. The aqueous extract (4.6%) had substantially more caryophyllene, a

compound with anti-inflammatory properties (Gushiken et al., 2022)—than the methanolic extract (3.4%). Additionally, the methanolic extract (5.8%) contained more of the chemical germacrene D (which may have antibacterial activity) than the aqueous extract (5.0%). Similar trends were observed for beta-selinene, which was higher in the methanolic extract (2.9%) compared to the aqueous extract (2.3%).

The aqueous extract had larger quantities of alpha-cadinol and delta-cadinene (1.1% and 0.8%, respectively) than the methanolic extract (0.8% and 0.6%, respectively), both of which had anti-inflammatory and antibacterial properties (Tripathi et al., 2022). With a reputation for being an antioxidant (Gulcin, 2020), epiglobulol was found in higher concentration in the methanolic extract (2.7%) compared to the aqueous extract (1.9%). The methanolic extract (2.4%) contained larger amounts of phytol, an antioxidant and antibacterial compound (Nwabiani et al., 2023), than the aqueous extract (1.7%).

There was 0.9% of luteol, a substance with anti-inflammatory and anticancer effects (Raju et al., 2023), in the methanolic extract and 0.5% in the aqueous extract. With a reputation for reducing cholesterol and reducing inflammation (Naqvi et al., 2019), stigmasterol was found in somewhat higher concentrations in the methanolic extract (1.2%) as opposed to the aqueous extract (1.0%). These findings demonstrate that these beneficial components, with the exception of alpha-thujene, alpha-cadinol, and delta-cadinene, are typically found in higher concentrations in the methanolic extract, indicating that methanol is a more efficient solvent for removing these phytochemicals from *Vernonia amygdalina L.* leaves.

3.4 Determination of Non-volatile Bioactive Substances using HPLC

A thorough profile of the non-volatile bioactive substances in the aqueous extract was given by the HPLC analysis. Table 2 presents the findings.

Table 2: Variations of Phytochemicals in Methanolic and Aqueous Extract by HPLC

Compound	Aqueous Extract (mg/g)	Methanolic Extract (mg/g)
Gallic acid	0.5 ± 0.05	0.8 ± 0.08
Chlorogenic acid	2.3 ± 0.15	3.1 ± 0.18
Caffeic acid	1.2 ± 0.09	1.5 ± 0.12
Rutin	4.2 ± 0.25	3.5 ± 0.20
Quercetin	2.1 ± 0.12	2.8 ± 0.15
Kaempferol	0.7 ± 0.04	1.0 ± 0.06

Luteolin	1.8 ± 0.10	2.4 ± 0.13
Apigenin	0.9 ± 0.06	1.2 ± 0.07
Naringenin	1.0 ± 0.08	1.3 ± 0.09
Catechin	1.5 ± 0.11	2.0 ± 0.14

From table 2, a significant variation in the amounts of several phenolic and flavonoid components were found between the aqueous and methanolic extracts of *Vernonia amygdalina L.* leaves, according to HPLC analysis. The methanolic extract (0.8 mg/g) contained greater amounts of gallic acid, a strong antioxidant (Hussein et al., 2020), than the aqueous extract (0.5 mg/g). Comparably, the methanolic extract (3.1 mg/g) contained higher chlorogenic acid, known for its anti-inflammatory and antioxidant qualities (Wang et al., 2021), than the aqueous extract (2.3 mg/g). Another potent antioxidant, caffeinic acid (Lopes et al., 2021), was found in greater concentrations in the methanolic extract (1.5 mg/g) than in the aqueous extract (1.2 mg/g).

The aqueous extract (4.2 mg/g) contained more rutin, a flavonoid with potent anti-inflammatory and antioxidant properties (Liu et al., 2023), than the methanolic extract (3.5 mg/g). With a wide range of health advantages, including anti-inflammatory and antioxidant properties (Qahl & Hamza, 2024), quercetin was found to be more concentrated in the methanolic extract (2.8 mg/g) than in the aqueous extract (2.1 mg/g). The methanolic extract (1.0 mg/g) had more kaempferol, an antioxidant and anticancer substance (Wang et al., 2019), than the aqueous extract (0.7 mg/g).

A molecule with potent anti-inflammatory and antioxidant properties, luteolin (Huang et al., 2023), was found in higher concentrations in the methanolic extract (2.4 mg/g) as opposed to the aqueous extract (1.8 mg/g). The methanolic extract (1.2 mg/g) had more apigenin, known for its anti-inflammatory, antioxidant, and anticancer effects (Kashyap et al., 2021) than the aqueous extract (0.9 mg/g). The methanolic extract (1.3 mg/g) contained higher naringenin, an antioxidant and anti-inflammatory compound (Li et al., 2023) than the aqueous extract (1.0 mg/g). The methanolic extract contained more catechin (2.0 mg/g) than the aqueous extract (1.5 mg/g), which is well-known for its antioxidant qualities (Grzesik et al., 2018).

Overall, these findings show that, in comparison to the aqueous extract, the methanolic extract of *Vernonia amygdalina L.* leaves has higher quantities of phenolic and flavonoid components with exception of rutin. This shows that methanol is a more efficient solvent for removing these bioactive substances. The methanolic extract may have more antioxidant and therapeutic potential due to the larger quantities of these components.

3.5 Determination of DPPH Radical Scavenging Activity

The investigation of the DPPH radical scavenging activity reveals clear differences in the antioxidant activity between the methanolic and aqueous extracts of *Vernonia amygdalina L.* leaves at varying doses (figure 5). The methanolic extract had greater scavenging activity (28.6%) than the aqueous extract (25.3%) even at the lowest concentration of 20 µg/mL. At every concentration level, the methanolic extract exhibits higher antioxidant activity, following a steady trend. The methanolic extract demonstrates 48.5% scavenging action at 50 µg/mL, but the aqueous extract only reaches 43.2%. The activity of the methanolic extract increases to 68.4% at 100 µg/mL, whilst the aqueous extract displays 61.7%. The methanolic extract exhibits 80.2% scavenging action at 150 µg/mL, while the aqueous extract shows 75.3%. The methanolic extract has the maximum scavenging activity at 90.5% at the highest concentration of 200 µg/mL, whereas the aqueous extract shows 85.4%.

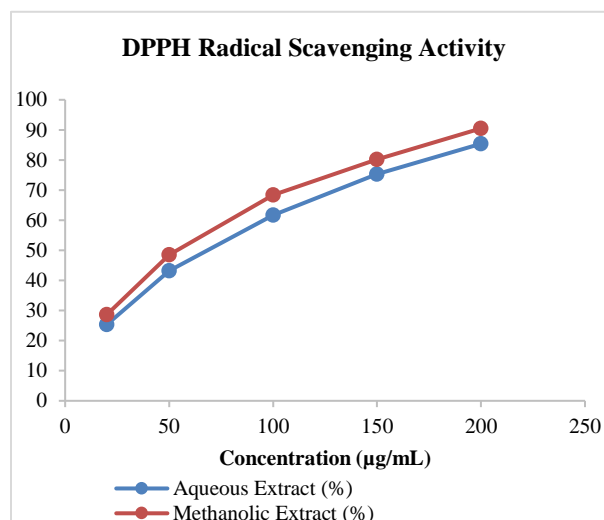


Figure 5: DPPH Radical Scavenging Activity

These findings imply that, at all tested concentrations, the methanolic extract has a higher potential for antioxidants than the aqueous extract. The methanolic extract's higher total phenolic and flavonoid content is consistent with its higher DPPH radical scavenging activity, suggesting that methanol is more efficient in extracting the bioactive ingredients from the bitter leaf that give rise to antioxidant activity. The trend of increasing scavenging activity at higher doses supports the idea that the

antioxidant components in both extracts are dose-dependent (Ali et al., 2024).

3.6 Determination of ABTS Radical Scavenging Activity

The ABTS radical scavenging activity assay shows that the antioxidant activity of the aqueous and methanolic extracts of *Vernonia amygdalina L.* leaf extracts vary significantly across different doses. At the lowest concentration of 20 $\mu\text{g/mL}$, the methanolic extract demonstrates higher scavenging action (34.7%) compared to the aqueous extract (30.2%) as delineated in figure 6. The methanolic extract shows increased antioxidant activity in a consistent manner at all concentration levels. At 50 $\mu\text{g/mL}$, the methanolic extract exhibits 56.8% scavenging activity while the aqueous extract shows 51.6%. The activity of the methanolic extract increases to 76.1% at 100 $\mu\text{g/mL}$, whereas the aqueous extract displays 70.5%. The methanolic extract has 88.4% scavenging activity at 150 $\mu\text{g/mL}$, while the aqueous extract shows 83.2%. The methanolic extract has the maximum scavenging action at 200 $\mu\text{g/mL}$, with 96.8%, whereas the aqueous extract shows 92.7%.

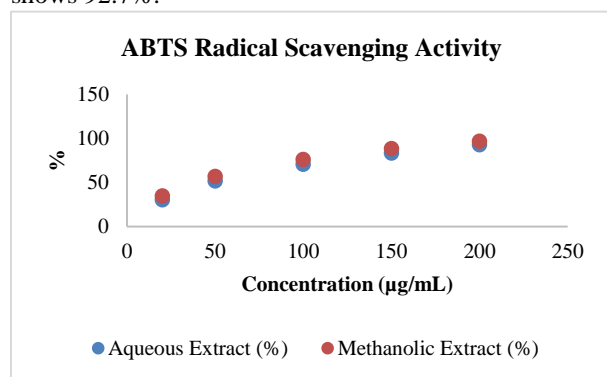


Figure 6: ABTS Radical Scavenging Activity

These results suggest that the methanolic extract has a stronger potential for antioxidants than the aqueous extract at all tested doses. The greater ABTS radical scavenging activity seen in the methanolic extract is consistent with its enhanced total phenolic and flavonoid content, which are known to contribute to antioxidant activity. More phytochemicals are solubilized by methanol, indicating that it is effective in extracting these advantageous compounds from the bitter leaf. The hypothesis that the antioxidant components in both extracts are dose-dependent is supported by the trend of increasing scavenging activity at higher dosages.

To sum up, the methanolic extract of *Vernonia amygdalina L.* leaves consistently displays stronger antioxidant activity compared to the aqueous extract

across all tested doses, according to the results of both DPPH and ABTS radical scavenging activity assays. With a peak of 90.5% at 200 $\mu\text{g/mL}$, the methanolic extract outperformed the aqueous extract in the DPPH experiment, scavenging more than the latter at every concentration level. Comparably, in the ABTS test, the aqueous extract obtained 92.7% antioxidant activity but the methanolic extract showed better antioxidant activity at all doses, reaching 96.8% at 200 $\mu\text{g/mL}$. These data show a close association between the DPPH and ABTS assay results, indicating that the methanolic extract consistently exhibits better antioxidant capacity than the aqueous extract. This is probably because the methanolic extract contains more phenolic and flavonoid components (Nazir, 2024).

4.0 CONCLUSION

In this work, methanolic and aqueous extracts of *Vernonia amygdalina L.* leaves were compared for their phytochemical content and antioxidant activity. The methanolic extract was discovered to have a higher concentration of bioactive components, including gallic acid, quercetin, caffeic acid, rutin, and chlorogenic acid, using sophisticated techniques like NMR, FTIR, HPLC, and GC-MS. Additionally, this extract showed increased antioxidant capacity in the ABTS and DPPH tests. Key phytochemicals such as caryophyllene, phytol, and germacrene D were found in higher concentrations in the methanolic extract as compared to the aqueous extract, according to GC-MS analysis. These results imply that methanol is more efficient at removing bioactive components, boosting the antioxidant capacity of the leaves, and suggesting possible use in pharmaceuticals and health supplements both commercially and therapeutically (Durumin-Iya., 2023). In order to optimize the health advantages of the plant, the study also emphasizes the necessity for additional investigation into the bioavailability, mechanism of action, and therapeutic efficacy of these bioactive components.

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COMPETING INTEREST

The authors of this research affirm that they have no conflicting interests in publishing it. The research was carried out impartially, free from any personal or financial connections that might have influenced the findings. The authors acknowledge all funding sources for this research and retain complete ownership over the primary data and materials used in this publication.



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