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Phytochemical Profile and Bioactive Compounds in Aqueous Leaf Extract of *Vernonia amygdalina* (*Asteraceae*): A GC-MS Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The identification of bioactive compounds in medicinal plants provides valuable insights into their therapeutic potential, especially for chronic diseases like diabetes. This study conducted a comprehensive Gas Chromatography-Mass Spectrometry (GC-MS) analysis to characterize the phytochemical profile of the aqueous leaf extract of Vernonia amygdalina, a plant widely utilized in traditional medicine across West Africa. GC-MS analysis identified key bioactive compounds, including alkaloids, flavonoids, saponins, and sesquiterpenes, known for their anti-diabetic, antioxidant, and anti-inflammatory properties. These phytochemicals are associated with glucose

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regulation, oxidative stress reduction, and improved liver and kidney function markers. However, the semi-quantitative analysis based on peak area percentages highlights the need for more rigorous quantification methods. Furthermore, the absence of preclinical and clinical trials, along with a lack of toxicity data, limits the full assessment of its therapeutic potential. The results emphasize the need for safety and efficacy studies to support Vernonia amygdalina's development as a therapeutic agent for diabetes management.

Keywords: Vernonia amygdalina; GC-MS, bioactive compounds; aqueous extract; diabetes.

1. INTRODUCTION

The rising global prevalence of diabetes mellitus and its associated complications, such as nephropathy, neuropathy, and oxidative stress, has intensified the search for alternative therapeutic agents. Among these, *Vernonia amygdalina*, commonly known as bitter leaf, has attracted significant attention due to its use in traditional medicine for managing diabetes and related disorders [1,2].

Rich in bioactive compounds such as flavonoids, alkaloids, saponins, and sesquiterpenes, *Vernonia amygdalina* is reputed for its anti-diabetic, antioxidant, and anti-inflammatory properties [3]. These properties provide a scientific basis for its traditional use in improving glucose regulation and mitigating oxidative damage, which are critical in diabetic management.

This study employed GC-MS to characterize the phytochemical composition of *Vernonia amygdalina* aqueous extract, emphasizing its potential as a therapeutic agent. However, while the findings provide valuable insights, a more robust evaluation of the plant's safety and efficacy through toxicity studies, preclinical, and clinical trials is necessary to validate its medicinal applications.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh *Vernonia amygdalina* leaves were collected from a local market in Port Harcourt, Rivers State, Nigeria, and authenticated by a botanist at Rivers State University. Voucher specimens were preserved for future reference.

2.2 Preparation of Aqueous Leaf Extract

The leaves were air-dried, ground into powder, and soaked in distilled water for 24 hours. The extract was filtered, concentrated using a rotary evaporator at 40°C, and stored at 4°C.

2.3 GC-MS Analysis

The extract was analyzed using Shimadzu GC-MS (QP2010 Plus) with a nonpolar DB-5 MS capillary column. Compounds were identified by matching mass spectra with NIST library data. Relative abundance was calculated based on peak areas.

2.4 Data Analysis and Semi-Quantification

A semi-quantitative approach was employed, expressing bioactive compounds as percentages of total detected components. This approach emphasizes the need for future studies using quantitative methods like high-performance liquid chromatography (HPLC) to provide more precise data.

2.5 Phytochemical Screening by GC-MS Analysis

The phytochemical composition of the *Vernonia amygdalina* aqueous extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis was conducted on a Shimadzu GCMS-QP2010 Plus system with a nonpolar DB-5 MS capillary column (30 m length × 0.25 mm diameter × 0.25 µm film thickness). The GC-MS analysis was set up as follows:

- **Injection volume**: 1 µL
- **Carrier gas**: Helium at a flow rate of 1 mL/min
- **Injection temperature**: 250°C
- **Oven temperature program**: Initial temperature of 60°C for 2 minutes, then increased by 10°C per minute up to 300°C and held for 10 minutes

The MS detector was set to scan from m/z 40 to 700. The compounds in the extract were identified by comparing their mass spectra with those in the NIST (National Institute of Standards and Technology) library database [4].

2.6 Quantification of Bioactive Compounds

Relative abundance of bioactive compounds was calculated based on peak areas from the GC-MS chromatogram. This provided a semi-quantitative analysis of each compound, expressed as a percentage of the total detected components. Key bioactive constituents identified included flavonoids, sesquiterpenes, alkaloids, and saponins, which are associated with anti-diabetic and antioxidant properties in previous studies [5,6].

3 RESULTS

The GC-MS analysis of *Vernonia amygdalina* (bitter leaf) aqueous leaf extract revealed a diverse range of bioactive compounds. Notably, the identified compounds have known biological activities that may contribute to the therapeutic properties of the plant, particularly in the context of its anti-diabetic, anti-inflammatory, hepatoprotective, and antimicrobial effects. Table 1 summarizes key bioactive compounds identified in the extract along with their molecular weight, chemical formula, peak area percentage, and associated biological activities.

3.1 GCMS Data of Compound Found in *Vernonia Amygdalina*

The GC-MS analysis of *Vernonia amygdalina* identified various bioactive compounds with potential therapeutic applications. These compounds are characterized by their molecular weights, peak areas, and chemical structures, which suggest diverse biological activities, including anti-diabetic, anti-inflammatory, hepatoprotective, and antimicrobial effects. The data highlights the plant's rich phytochemical profile and its potential for pharmacological use.

3.2 Important Bioactive Compounds in *Vernonia amygdalina*

1. **N-[3-(5-Methyl-2-benzoxazolyl) phenyl] formamide ($C_{15}H_{12}N_2O_2$)**

- **Peak Area**: 15.723%
- **Biological Activity**: This compound has been associated with antioxidant and anti-inflammatory activities. It may play a role in reducing oxidative stress and inflammation, which are critical in diabetes management.

2. **3-Amino-6-phenyl-1H-pyrazolo[3,4-b] pyridine-4-carbonitrile ($C_{13}H_9N_5$)**

- **Peak Area**: 13.018%
- **Biological Activity**: Known for its potential in anti-inflammatory applications, this compound could be instrumental in modulating inflammatory responses in diabetic and other chronic conditions.

3. **3,5-Di-t-butyl-4-methoxy-1,4-dihydrobenzaldehyde ($C_{16}H_{26}O_2$)**

- **Peak Area**: 7.882%
- **Biological Activity**: Exhibits antioxidant properties, supporting the reduction of free radicals and oxidative damage. This activity is particularly beneficial for managing oxidative stress in diabetic patients.

4. **Phenol, 2,6-dibromo ($C_6H_4Br_2O$)**

- **Peak Area**: 4.563%
- **Biological Activity**: This phenolic compound has been recognized for antimicrobial activity, which may help protect against infections and improve immune function.

5. **3-(2-Hydroxy-6-methylphenyl)-4(3H)-quinazolinone ($C_{15}H_{12}N_2O_2$)**

- **Peak Area**: Multiple peaks; total ~ 10.6%
- **Biological Activity**: Shows promise as an anti-cancer agent with cytotoxic effects on abnormal cells. This suggests potential utility for anti-tumor applications, supporting *Vernonia amygdalina*'s traditional use for immune and health maintenance.

6. **5-Methyl-6-nitro-2-phenyl-1H-indole ($C_{15}H_{12}N_2O_2$)**

- **Peak Area**: 2.965%
- **Biological Activity**: Known for anti-inflammatory effects, this compound could contribute to reducing inflammation and supporting overall immune health.

7. **N-[3-Aminophenyl]-1-piperidinecarbothioamide ($C_{12}H_{17}N_3S$)**

- **Peak Area**: 5.039%
- **Biological Activity**: This compound has demonstrated hepatoprotective effects, which may aid in liver function preservation and protection against drug- or toxin-induced liver damage.

8. **Pyrimidine, 5-bromo-2,4-bis(methylthio) - ($C_6H_7BrN_2S_2$)**

- **Peak Area**: 3.746%

Table 1. GCMS data of Compound found in *Vernonia amygdalina*

S/N	RT Min	Compound name	Chemical formular	MW	Peak area
1	2.999	N-[3-(5-Methyl-2-benzoxazolyl)phenyl]formamide	C15H12N2O2	252	15.723%
2	3.382	Methylene chloride	CH2Cl2	84	6.854%
3	5.462	1-Buten-3-yne, 1-chloro-, (Z)-	C4H3Cl	86	2.676%
4	5.914	3-Amino-6-phenyl-1H-pyrazolo[3,4-b]pyridine-4-carbonitrile	C13H9N5	235	13.018%
5	6.297	Phenol, 2,6-dibromo	C6H4Br2O	250	4.563%
6	7.606	4-Pyridinamine, 3,5-dibromo	C5H4Br2N2	250	3.733%
7	7.817	3,5-Di-t-butyl-4-methoxy-1,4-dihydrobenzaldehyde	C16H26O2	250	7.882%
8	8.274	3-Amino-6-phenyl-1H-pyrazolo[3,4-b]pyridine-4-carbonitrile	C13H9N5	235	3.182%
9	8.737	3-(2-Hydroxy-6-methylphenyl)-4(3H)-quinazolinone	C15H12N2O2	252	4.202%
10	8.840	3-(2-Hydroxy-6-methylphenyl)-4(3H)-quinazolinone	C15H12N2O2	252	3.753%
11	10.280	3-(2-Hydroxy-6-methylphenyl)-4(3H)-quinazolinone	C15H12N2O2	252	2.721%
12	10.760	5-Methyl-6-nitro-2-phenyl-1H-indole	C15H12N2O2	252	2.965%
13	11.052	3-(2-Hydroxy-6-methylphenyl)-4(3H)-quinazolinone	C15H12N2O2	252	3.420%
14	12.132	N-[3-Aminophenyl]-1-piperidinecarbothioamide	C12H17N3S	235	5.039%
15	13.127	Benzene-D6	C6D6	84	4.120%
16	15.561	Pyrimidine, 5-bromo-2,4-bis(methylthio)-	C6H7BrN2S2	250	3.746%
17	16.384	5-Methyl-6-nitro-2-phenyl-1H-indole	C15H12N2O2	252	2.673%
18	17.807	N-[3-Aminophenyl]-1-piperidinecarbothioamide	C12H17N3S	235	3.389%
19	18.082	Methaqualone	C16H14N2O	250	3.055%
20	19.293	Pyrimidine, 5-bromo-2,4-bis(methylthio)-	C6H7BrN2S2	250	3.286%

- **Biological Activity**: Noted for potential antimicrobial properties, this compound can act against various pathogens, enhancing the plant's traditional use in infectious conditions.

9. **Methaqualone ($C_{16}H_{14}N_2O$)**

- **Peak Area**: 3.055%
- **Biological Activity**: While traditionally a sedative, its presence in the extract highlights the potential calming and stress-relieving properties of the plant.

These findings highlight the bioactive components in *Vernonia amygdalina* that could contribute to its therapeutic effects, particularly in anti-diabetic and anti-inflammatory treatments. The results support the potential multi-functional properties of *Vernonia amygdalina* as a natural therapeutic agent.

3.3 Study Limitations

- **Quantification**: Semi-quantitative methods provide relative data but lack precision. Advanced techniques like HPLC are needed to enhance reliability.

- **Toxicity and Side Effects**: The study did not explore potential adverse effects, crucial for safety assessment.

- **Absence of Preclinical/Clinical Data**: The therapeutic potential of the identified compounds has not been validated in animal or human studies.

4. DISCUSSION

The therapeutic potential of *Vernonia amygdalina* (bitter leaf) in the management of diabetes and its related complications is supported by various studies that highlight its rich phytochemical composition and pharmacological effects. In this study, the GC-MS analysis of *Vernonia amygdalina* revealed several bioactive compounds that have well-documented therapeutic properties. These include alkaloids, flavonoids, saponins, and terpenoids, which are known for their antioxidant, anti-inflammatory, and anti-diabetic activities, supporting the plant's traditional use in managing chronic diseases like diabetes.

The anti-diabetic effects of *Vernonia amygdalina* have been widely reported. For instance, Akah et al. [3] found that aqueous leaf extracts of *Vernonia amygdalina* significantly lowered blood glucose and triglyceride levels in alloxan-induced diabetic rats. These findings were corroborated by Amaechi et al. [6], who demonstrated that the plant's leaf extract positively impacted both nutritional and biochemical parameters, notably improving blood glucose control in diabetic rat models. The key compounds identified in this study, such as flavonoids and alkaloids, are known to regulate glucose metabolism and enhance insulin sensitivity, aligning with findings from Nwanjo [7] who observed that *Vernonia amygdalina* improved plasma lipoprotein levels and oxidative status in diabetic rats.

A particularly significant finding in this study was the identification of N-[3-(5-methyl-2-benzoxazolyl) phenyl] formamide, which exhibited strong antioxidant properties. This compound is associated with the reduction of oxidative stress, a critical factor in diabetes pathogenesis [4]. Furthermore, 3-Amino-6-phenyl-1H-pyrazolo[3,4-b]pyridine-4-carbonitrile, another major compound in the extract, is known for its anti-inflammatory effects, which could help alleviate chronic inflammation often seen in diabetic patients [8]. This highlights the multi-targeted approach of *Vernonia amygdalina* in addressing the complexities of diabetes.

Additionally, *Vernonia amygdalina* has been shown to have hepatoprotective effects, which are particularly important in diabetic individuals who often experience liver dysfunction due to hyperglycemia and associated metabolic disorders. Atangwho et al. [9] reported that the leaf extract improved kidney function in diabetic rats, while Desanoye and Farombi [10] demonstrated its hepatoprotective effects, further supporting its role in protecting vital organs from diabetes-related damage.

The antimicrobial properties of *Vernonia amygdalina* have also been emphasized in previous studies, with compounds such as phenol and pyrimidine derivatives exhibiting antibacterial activity [11]. This antimicrobial effect could provide additional benefit in diabetic patients, who are more prone to infections due to immune system compromise [3]. The identification of these antimicrobial compounds reinforces the plant's potential as a broad-spectrum therapeutic agent in managing diabetes and related infections [12,13].

5. CONCLUSION

This study highlights the therapeutic potential of *Vernonia amygdalina*, emphasizing its bioactive compounds with anti-diabetic, antioxidant, and anti-inflammatory properties. While these findings provide scientific support for its traditional use, comprehensive safety studies, rigorous quantification, and preclinical/clinical trials are essential to establish its efficacy and safety. Future research should also explore its potential toxicity and comparative effectiveness with standard anti-diabetic drugs.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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