**Bioactive Analysis of *Anthocleista vogelii* Leaves for Therapeutic Potential in Haemolytic Anaemia**

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ABSTRACT

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| **Aims**: The aim of the study was to evaluate the proximate and mineral composition and identify bioactive compounds in the methanol leaf extract of *Anthocleista vogelii* using gas chromatography-mass spectrophotometer (GC-MS analysis), with a focus on its potential antioxidant, antimicrobial, and anti-inflammatory properties. The study also sought to highlight the plant's therapeutic potential, particularly in mitigating oxidative stress and supporting erythropoiesis.  **Study design:** Plant leaf samples were collected and authentication at the herbarium, followed by drying and processing of the leaves for proximate, mineral, and (GC-MS) analysis.  **Place and Duration of Study:** The study was conducted at Delta State University, Abraka, Nigeria. The leaves were harvested and analyzed over a period of three weeks, starting from the time of collection and drying to the completion of extraction and analysis.  **Methodology:** The study employed a quantitative experimental design aimed at analyzing the proximate and mineral composition and bioactive compounds in *Anthocleista vogelii* leaves using standard analytical methods.  **Results:** Proximate analysis revealed high nitrogen-free extract (44.44 ± 1.09 %), crude protein (15.14 ± 1.18 %), ether extract (14.77 ± 0.44 %), moisture content (11.93 ± 1.30 %), ash content (8.30 ± 1.26 %), and crude fibre (5.40 ± 0.53 %) while mineral analysis indicated significant concentration of potassium (18.35 ppm), sodium (9.52 ppm), and selenium (1.33 ppm). GC-MS identified compounds such as Vitamin E, catechol, and n-hexadecanoic acid with antioxidant, antimicrobial, and anti-inflammatory properties. The identified bioactive compounds exhibit synergistic effects, contributing to the extract's therapeutic potential. Selenium and zinc were highlighted for their roles in mitigating oxidative stress and supporting erythropoiesis. *Anthocleista vogelii* leaf extract is a rich source of bioactive compounds with significant antioxidant, antimicrobial, and therapeutic properties.  **Conclusion:** The findings support its traditional use in managing oxidative stress-related conditions and provide a foundation for further pharmacological studies. |

*Keywords: [*Proximate; mineral; bioactive compounds; leaf extract*]*

1. INTRODUCTION

*Anthocleista vogelii*, a medicinal plant widely utilized in traditional medicine, has gained scientific interest due to its rich bioactive composition and potential therapeutic benefits. The exploration of plant extracts for their therapeutic, nutritional, and industrial applications has been a focal point of scientific research. The research conducted by Olubomehin, Faponle, Ajaiyeoba & Abo (2022) and Anyanwu, Onyeneke, Ofoha, Rauf, Usunobun & Nisar-ur-Rehman (2018) highlighted the diverse range of ailments for which these plants are employed. *Anthocleista* species are utilized in the treatment of eye-related conditions, showcasing their potential in addressing ophthalmic troubles. The medicinal properties of *Anthocleista* extend to respiratory health, making it a valuable resource in managing pulmonary issues. Traditional medicine employs *Anthocleista* species in the treatment of fevers, suggesting its antipyretic potential. The plants have been researched for their role in managing hypertension and diabetes, indicating a possible avenue for the development of herbal remedies for these chronic conditions (Ngwoke, Akwagbulam, Erhirhie, Ajaghaku, Okoye & Esimone, 2018; Oredeko, Omeiza, Ben-Azu, Ogiogio, Falade, Aderibigbe, & Gbotosho, 2023).

To maximize the utility of these extracts, comprehensive analytical techniques such as proximate analysis, mineral profiling, and Gas Chromatography-Mass Spectrometry (GC-MS) are indispensable. These methods provide vital insights into the chemical composition, nutritional value, and bioactive compounds present in plant extracts.

This study investigates the proximate and mineral composition, extract yield, and chemical profiling of *Anthocleista vogelii* leaf extract, focusing on its antioxidant, antimicrobial, and anti-inflammatory properties. Employing methanol as the extraction solvent, the study leverages Gas Chromatography-Mass Spectrometry (GC-MS) analysis to identify bioactive compounds, emphasizing their roles in mitigating oxidative stress.

2. material and methods

**2.1 Collection of Plant Material and Identification**

*Anthocleista vogelii* leaves were harvested from Site 3 Delta State University Abraka, Delta State, Nigeria with longitude 5.7919°N and latitude 6.0987°E, identified and authenticated at the herbarium section of the Department of Botany, Delta State University, Abraka– Nigeria and the voucher sample was deposited in the herbarium of the University with voucher numbers UBH-A258.

**2.2 Extract Preparation**

The Fresh leaves of *Anthocleista vogelii* were washed and air dried for three weeks and then reduced to coarse powder. 100g of coarsely powdered leaf was extracted with 500ml of methanol solvents using cold maceration for 48 hours. The extract was then filtered using Whatman filter paper. The resulting extract was then concentrated at 50oC in a rotary evaporator for 2hr and it was then transferred to a water bath maintained at 50oC and evaporated to dryness to yield a dark brown mass. The obtained extract was put in a glass container and stored at 4oC until when required for use.

**2.3 Proximate/Mineral/GC-MS Analysis**

Proximate analysis was carried out using the methods described by Isikhuemen, Ogbomwan & Efenudu, (2020) and Ganogpichayagrai & Suksaard (2020). Mineral analysis was carried out on the pulverized plant sample using standard procedure with an atomic absorption spectrophotometer and flame photometer (AOAC, 1984). Gas Chromatography/Mass Spectrometry (GC/MS) techniques was carried out using method decried by Zhao et al., (2011). Values are reported as Mean ± Standard deviation.

3. results and discussion

**3.1 Proximate Analysis**

Dried sample had a low moisture content and different macromolecules in their respective percentages as indicated in **table 1** Properly dried plant samples retain more active ingredients (Babu, Kumaresan, Raj, & Velray, 2024), enhancing their effectiveness in treating haemolytic anaemia; ash content indicates the total mineral content of the plant. Crude protein content represents the plant's amino acid profile, amino acids are necessary for the synthesis of haemoglobin and other proteins involved in red blood cell production. Adequate protein concentration observed can support the body’s capacity to generate new red blood cells. While fiber itself may not directly treat haemolytic anaemia, it aids in overall digestive health, potentially improving nutrient absorption, including iron and other minerals vital for red blood cell production. Crude fat content provides information on the lipid profile of the plant and is important for maintaining cell membrane integrity, including red blood cells. Carbohydrates provide energy, which is crucial for the metabolism and function of all cells, including those involved in erythropoiesis (red blood cell production)

Table 1: Proximate Analysis of Methanol Extract of *Anthocleista vogelii*

|  |  |
| --- | --- |
| Proximate | % |
| Moisture Content | 11.93 ± 1.30 |
| Crude Protein | 15.14 ± 1.15 |
| Ash Content | 8.30 ± 1.26 |
| Ether Extract | 14.77 ± 0.44 |
| Crude Fibre | 5.40 ± 0.53 |
| Nitrogen Free Extract | 44.44 ± 1.09 |

***Keys****: Data reported as mean + standard deviations of triplicate determinations*

**3.2 Mineral Content**

The mineral analysis of *Anthocleista vogelii* (**table 2**) indicating the presence of selenium suggests significant therapeutic potential, especially in the context of haemolytic anaemia induced by phenylhydrazine. Plants often have selenium concentrations in the range of 0.1 to 2 ppm (National Research Council (US) Subcommittee on Selenium, 1983), so the concentration for selenium in this extract appears to be within the expected range. Selenium is a cofactor for Glutathione Peroxidase (GPx), an enzyme that reduces hydrogen peroxide and lipid peroxides, converting them into water and non-toxic substances (Lara et al., 2024). By doing so, GPx prevents oxidative damage to red blood cell membranes caused by phenylhydrazine-induced oxidative stress. Another selenium-dependent enzyme, thioredoxin reductase, plays a role in reducing oxidative stress by maintaining the redox balance within cells. This enzyme helps protect red blood cells from oxidative damage, promoting their integrity and longevity; selenium-containing enzymes prevent lipid peroxidation. Selenium is crucial for the optimal function of the immune system (Rotruck, Pope, Ganther & Hoekstra, 2009). It supports the activity of various immune cells, including macrophages and lymphocytes, which can help manage the inflammatory response associated with haemolytic anaemia. Selenium helps reduce the production of pro-inflammatory cytokines and promotes the production of anti-inflammatory cytokines (Mal'tseva, Goltyaev, Turovsky, & Varlamova, 2022). This balance helps minimize inflammation-induced damage to red blood cells, a common issue in haemolytic anaemia. Selenium is involved in DNA synthesis and repair mechanisms, which are crucial for the proliferation and maturation of red blood cells: Selenium is a component of selenoproteins, which play roles in DNA synthesis and repair. These proteins ensure that erythroid progenitor cells in the bone marrow can proliferate and mature into functional red blood cells, aiding recovery from haemolytic anaemia. Selenium contributes to the detoxification of harmful substances that may accumulate in red blood cells and also helps regulate the concentration of other essential metals within the body, ensuring a balanced environment for red blood cell production and function.

Zinc is essential for the activity of ALA synthase (δ-aminolevulinic acid synthase), which catalyzes the first step in the haem synthesis pathway (Kiening & Lange, 2022). Adequate zinc concentrations ensure efficient haem production, which is vital for forming functional haemoglobin in red blood cells. In the case of phenylhydrazine-induced haemolytic anaemia, enhancing haem synthesis can help replenish the destroyed red blood cells. Zinc is a component of SOD, an antioxidant enzyme that protects red blood cells from oxidative damage. Phenylhydrazine induces oxidative stress, leading to the destruction of red blood cells. Zinc, by supporting SOD activity helps neutralize superoxide radicals, thereby protecting red blood cells from further damage and promoting their longevity (Delesderrier, Curioni, Omena, Macedo, Cople-Rodrigues & Citelli, 2020). Zinc supports the rapid cell division and differentiation of erythroid progenitor cells in the bone marrow (López, Moten, M & Chen, 2023). In the context of phenylhydrazine-induced haemolysis, zinc can facilitate the production of new red blood cells to replace those that have been prematurely destroyed. This process is critical for the recovery from haemolytic anaemia. Zinc helps regulate the synthesis of globin, the protein component of haemoglobin. Adequate zinc concentrations ensure that haemoglobin production is efficient, allowing new red blood cells to carry sufficient oxygen. This is particularly important in managing haemolytic anaemia, where maintaining high haemoglobin levels is crucial. Zinc helps maintain a balanced immune response, preventing excessive inflammation that can negatively impact erythropoiesis. Chronic inflammation can lead to anaemia of chronic disease, where red blood cell production is impaired. By modulating the immune response, zinc supports the healthy production of red blood cells. Zinc’s anti-inflammatory properties can protect red blood cells from damage due to immune-mediated destruction, which can be exacerbated in haemolytic conditions. Zinc fingers are structural motifs in many transcription factors that bind to DNA and regulate gene expression. These transcription factors control the expression of genes necessary for the growth and differentiation of erythroid cells. By influencing gene expression, zinc ensures the efficient production and maturation of red blood cells.

Table .2: Minerals Content of *Anthocleista vogelii* Methanol Extract

|  |  |
| --- | --- |
| Minerals | Concentration (ppm) |
| Se | 1.3332 |
| Zn | 0.7517 |
| Fe | 0.8323 |
| Ca | 0.0711 |
| Cu | 0.1303 |
| Co | 0.2168 |
| K | 18.3529 |
| Mn | 0.1447 |
| Mg | 11.8161 |
| Na | 9.5172 |

**3.3 GC-MS Analysis of *Anthocleista vogelii* Leaf Extract**

The compounds identified in the methanol leaf extract of *Anthocleista vogelii* were Vitamin E, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxyl-6-methyl, Catechol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, n-Hexadecanoic acid, 9,12,15-octadecatrienoic acid, (Z,Z,Z), Octadecanoic acid, Erythritol, 2-Butenoic acid, 2-methyl-, methyl ester, (Z), Cyclobutane-1,1-dicarboxamide, N,N-di-benzoyloxy, 1,2,3,4-butanetetrol [S-(R,R)], 4-O-Methylmannose, Card-20(22)-enolide, 3-[(2,6-dideoxy-4-O-β-D-glucopyranosyl-3-O-methyl-β-D-ribo-hexopyranosyl)oxy]-5,14-dihydroxy-19-oxo-,(3β,5β),4-C-methyl-myo-inositol, N-Serylserine, 2,2,4-Trimethyl-3-pentanol, Phenol, 3-isopropoxy-5-methyl, 4,8-Decadien-3-ol, 5,9-dimethyl-5-bromopentanoic acid and vanillin (**table 3**).

Vitamin E is well-known for its role in protecting cells from oxidative stress by scavenging free radicals (Idan, Al-Marzoqi & Hameed 2015). 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxyl-6-methyl is known for its antioxidant properties, primarily through its ability to chelate metal ions and scavenge free radicals. Additionally, it can bind to metal ions like iron and copper, which catalyze the formation of ROS through Fenton reactions, thereby reducing the overall oxidative stress in the system. The molecule also possesses anti-inflammatory, antimicrobial, and antiproliferative properties (Sgopalakrishnan & Vadivel, 2011; Rane & Anusha, 2012; Sirigiri & Kandru, 2017; Kumar, Kumaravel & Lalitha, 2010; Yu, Zhao, Liu, Zeng & Hu, 2013). Catechol is known for its antioxidant activities (Razaviamri, Wang, Liu, & Lee, 2021). Compounds like 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol and n-Hexadecanoic acid have been reported to possess anti-inflammatory properties. Phenylhydrazine-induced toxicity often results in inflammation and oxidative stress. The anti-inflammatory effects of these compounds can help reduce inflammation, thereby providing a protective effect against the induced toxicity (Kala & Ammani, 2017; Ashwathanarayana & Raja, 2017; Sgopalakrishnan & Vadivel, 2011). Compounds such as 9,12,15-octadecatrienoic acid, (Z,Z,Z) and Octadecanoic acid have been implicated in maintaining membrane integrity and reducing oxidative stress. 1,2,3,4-butanetetrol [S-(R,R)], also known as erythritol, has been found to be effective in promoting tooth mineralization (Iwueke, Ejekwumadu, Chukwu, Nwodu & Akalonu, 2020). Furthermore, it exhibits in *vivo* antioxidant properties and helps prevent vascular damage caused by hyperglycemia (den Hartog et al., 2010). The compound Cyclobutane-1,1-dicarboxamide, N,N'-dibenzoyloxy, demonstrates a range of properties, including anaphylactic, anti-tumor, Arylamine-N-Acetyltransferase-Inhibition, decreased norepinephrine production, GABAergic effects, increased natural killer (NK) cell activity, inhibition of Tumor Necrosis Factor (TNF) production, myo-neurostimulation, NADH-Oxidase inhibition, and NADH-Ubiquinone-Oxido-reductase inhibition (Aswaran & Raman, 2014). Arylamine N-acetyltransferases (NAT) plays a crucial role in detoxifying and activating various aromatic amine drugs and carcinogens (Hernández-González, Herrera-Vargas, Martínez-Leija, Zavala-Reyes & Portales-Pérez, 2022). This molecule likely serves to protect the body from inhaled allergic agents, enhance neurotransmission of allergic signals, clear respiratory pathways of cellular debris, and protect lung tissues from oxidative bursts. The TNF inhibitor activity of the molecules in the plant can be beneficial for treating inflammatory reactions in the respiratory tract, which are associated with autoimmune and immune-mediated disorders. This is particularly valuable since many commercially available TNF inhibitors have significant side effects (Lopetuso, Cuomo, Mignini, Gasbarrini & Papa, 2023). The NADH-Ubiquinone-Oxido-reductase inhibition may help control bacterial infections in the respiratory tract. Compounds containing a 2-benzylbenzofuran moiety exhibit a wide range of pharmacological activities, including antioxidants, anti-inflammatory, antiangiogenic, and antiviral effects (Qin, Vo, Nakhai, Andersson & Elofsson, 2017). Additionally, 3-ethyl-4-methyl-1H-pyrrole-2,5-dione possesses antimicrobial properties (Paprocka, Pazderski, Mazur, Wiese-Szadkowska, Kutkowska, Nowak & Helmin-Basa 2022). Indolizine derivatives with various functional groups have applications in pharmaceuticals, demonstrating anti-inflammatory, antioxidant, antifungal, and antibacterial activities (Vemula, Vurukonda & Bairi, 2011; Teklu, Gundersen, Larsen, Malterud & Rise 2005; Gundersen, Negussie, Rise & Østby, 2003; Pearson & Guo, 2001; Olejníková, Birošová, Švorc, Vihonská, Fiedlerová, Marchalín & Šafá, 2015). Isosorbide dinitrate has a relaxing effect and can be used to treat chronic congestive heart failure and cardiac edema (Sosa, Bagi & Hameed, 2016). Phenolic vanillin not only serves as a flavoring agent in food but also possesses pharmacological properties, acting as an antioxidant and inhibiting bacterial pathogenicity, especially against gram-negative bacteria *P. aeruginosa* (Bezerra et al., 2017; Dua et al., 2023). Trans-cinnamic acid (tCA) exhibits a broad spectrum of biological activities, including antioxidant, anti-inflammatory, and anticancer properties (Zhu, Shang, Li & Zhen, 2016). 4-O-methylmannose demonstrates antibacterial properties (Kumar & Bhaskar, 2012; Suman, Chakkaravarthi & Elangomathavan 2013). Card-20(22)-enolide,3-[(2,6-dideoxy-4-O-β-D-glucopyranosyl-3-O-methyl-β-D-ribohexopyranosyl)oxy]-5,14-dihydroxy-19-oxo-,(3β,5β), also known as periplocoside, glucoperiplocymarin, or periplocin, has been found to prevent the proliferation of human liver cancer cells, halt cell cycle progression, induce apoptosis, and offer potential therapeutic benefits for various cancers (Lu, Song, Yang, Zhao, Zhang, Yang & Kang, 2014). It may also inhibit lung cancer growth, suppress Akt/Erk signaling pathways, block the G0/G1 cell cycle phase, and promote wound healing (Zhao, Han, Dai, Wei, Xiang, Wang & Shan, 2021; Lu et al., 2010). 4-C-methyl-myo-inositol is believed to hyper-stimulate the ovaries, contributing to the plant's traditional use for female fertility remedies.

The combined presences of these compounds in the methanol leaf extract likely results in a synergistic effect, enhancing the overall protective impact against phenylhydrazine-induced toxicity. The complex interaction between these bioactive molecules may amplify their individual effects, providing a more robust defence mechanism against oxidative stress and inflammation.

Table 3: GC-MS Analysis of *Anthocleista vogelli* Leave Extract

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S/N | Chemical Name | Rt (Min) | Area % | Mass (g/mol) |
|  | Erythritol | 3.236 | 0.13 | 122.11 |
|  | 2-butenoic acid, 2-methyl-, methyl ester, (z) | 3.946 | 0.14 | 114.14 |
|  | 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 5.279 | 0.18 | 144.13 |
|  | Cyclobutane-1,1-dicarboxamide, N,N di-benzoyloxy | 5.531 | 0.11 | 382.4 |
|  | Catechol | 5.914 | 0.13 | 142.18 |
|  | 2,3-dihydrobenzofuran | 6.16 | 0.51 | 120.15 |
|  | 3-ethyl-4-methyl-1H-pyrrole-2,5-dione, | 6.355 | 0.16 | 157.17 |
|  | Ethane, 1,2-bis(methylthio) | 7.030 | 0.02 | 122.25 |
|  | Indolizine | 7.121 | 0.21 | 117.15 |
|  | isosorbidedinitrate | 7.442 | 0.08 | 236.14 |
|  | Vanillin | 8.317 | 0.11 | 152.15 |
|  | Trans-cinnamic acid | 8.563 | 0.24 | 148.16 |
|  | 1,3,4-thiadiazol-2-amine | 9.015 | 0.30 | 101.13 |
|  | Isopulegol | 9.902 | 0.06 | 154.25 |
|  | Beta-d-glucopyranose,1-thio-,1- (n-hydroxyl-5-(methylthio)pentanimidate | 10.44 | 0.24 | 341.44 |
|  | Benzaldehyde, 4-hydroxy-3,5-dimethoxy | 10.972 | 0.14 | 182.17 |
|  | 4-((1e)-3-hydroxy-1-propenyl)-2-methoxyphenol | 11.693 | 0.73 | 180.201 |
|  | 4-((1E)-3-Hydroxy-1-propenyl)-2- methoxyphenol | 11.693 | 2.09 | 180 |
|  | 4-c-methyl-myo-inositol | 11.802 | 19 | 194.18 |
|  | 4-O-Methylmannose | 11.905 | 1.438 | 194 |
|  | Card-20(22)-enolide, 3-[(2,6-dideoxy-4-O-β-D-glucopyranosyl-3-O-methyl-β-D-ribo-hexopyranosyl)oxy]-5,14-dihydroxy-19-oxo-,(3β,5β) | 12.014 | 11.690 | 696.82 |
|  | Phenol, 3-isopropoxy-5-methyl | 12.185 | 2.68 | 166.222.68 |
|  | N-hexadecanoic acid | 13.58 | 15.14 | 256.42 |
|  | 4-ethyl phenol | 13.936 | 0.25 | 122.16 |
|  | Vitamin E | 15.830 | 1.78 | 430.71 |
|  | Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- | 16.488 | 0.72 | 290.5 |
|  | 2(3h)-furanone | 18.686 | 0.05 | 84.07 |
|  | Cyclobutanone, 2-methyl-2-oxiranyl | 19.075 | 0.04 | 126.15 |
|  | 1-methylene-2b-hydroxymethyl-3,3-d imethyl-4b-(3-methylbut-2-enyl)-cyclohexane | 20.087 | 0.04 | 222.37 |
|  | Pseudosolasodinediacetate | 20.436 | 0.10 | 499.72 |

4. Conclusion

Proximate and mineral analysis *of Anthocleista vogelii* leaf extract reveals nutritional and therapeutic potential. High fiber supports digestion and nutrient absorption, while crude fat and protein contribute to cellular integrity and metabolic functions. GC-MS analysis identifies bioactive compounds with antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties.

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