



Phytochemical Composition, Nutritional Value, and Antioxidant Properties of *Anthocleista djalensis*

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Abstract

Background and aims: Traditional medicinal plants represent a vital source of bioactive compounds with therapeutic potential. *Anthocleista djalensis*, a member of the Gentianaceae family, is extensively utilized in African traditional medicine for the treatment of diabetes, malaria, hypertension, and gastrointestinal disorders. This study aims to investigate the phytochemical composition, nutritional profile, and antioxidant properties of the stem bark of *Anthocleista djalensis* in order to provide scientific validation for its traditional applications and to explore potential modern uses.

Methods: The stem bark of *Anthocleista djalensis* was collected from Benin City, Nigeria, and authenticated for accuracy. Aqueous extraction of the plant material was conducted using a freeze-drying method to preserve the bioactive compounds. Phytochemical screening was performed to identify the presence of tannins, flavonoids, alkaloids, saponins, and phenolics. Proximate analysis was conducted to determine the moisture, protein, fat, fiber, ash, and carbohydrate content of the stem bark. The antioxidant activity was evaluated using the DPPH radical scavenging assay, with results compared to those of Vitamin C as a standard reference.

Results: The qualitative screening of *Anthocleista djalensis* stem bark revealed the presence of key phytochemicals, including alkaloids, saponins, tannins, flavonoids, and phenols. Proximate analysis indicated the composition of the bark as follows: $6.2 \pm 0.025\%$ protein, $37 \pm 1.6\%$ fat, $0.11 \pm 0.010\%$ fiber, $9.3 \pm 0.19\%$ ash, $47 \pm 1.8\%$ carbohydrate, and $25 \pm 0.91\%$ moisture. Antioxidant activity, measured using the DPPH radical scavenging assay, demonstrated a significant dose-dependent free radical scavenging effect, yielding an IC_{50} of $36.25 \pm 1.03 \mu\text{g/mL}$. This was compared to ascorbic acid, which exhibited an IC_{50} of $21.87 \pm 0.92 \mu\text{g/mL}$, with a statistically significant difference noted ($p < 0.05$).

Conclusion: The findings from this study support the traditional medicinal use of *Anthocleista djalensis* and underscore its potential for nutraceutical and pharmaceutical applications. The presence of various phytochemicals and notable antioxidant activity indicates that this plant may serve as a valuable source of bioactive compounds. Further research aimed at isolating and characterizing these bioactive constituents could pave the way for the development of novel therapeutic agents, enhancing our understanding of their mechanisms and potential health benefits.

Keywords: *Anthocleista djalensis*, Phytochemicals, Nutraceuticals, Traditional medicine, Antioxidant activity

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Introduction

Natural products are fundamental to drug discovery and public health initiatives due to their structural diversity and extensive range of bioactivities (1–5). Within this field, the genus *Anthocleista* (Gentianaceae)—which includes a small group of shrubs and trees found primarily in tropical Africa, Madagascar, and the Comoros—has attracted significant ethnopharmacological interest. Notably, *Anthocleista djalensis* A. Chev. is commonly used in West Africa for treating various health conditions, such as metabolic, infectious, and inflammatory

diseases, including diabetes, malaria, hypertension, and gastrointestinal disorders (6–9). Preliminary pharmacological studies have shown that extracts from different parts of *Anthocleista djalensis* exhibit a range of beneficial activities, including hypoglycaemic, antiparasitic, antimicrobial, antiproliferative, and fertility-enhancing effects (10–17).

Despite the promising findings regarding *Anthocleista djalensis*, the current understanding of this species is still quite fragmented. Firstly, the reported phytochemical profiles of *Anthocleista djalensis* vary significantly,

which can be attributed to differences in geographic origin, climatic conditions, and extraction methods used in various studies (18–20). Secondly, although compounds such as tannins, phenolics, saponins, and alkaloids have been identified, there is a lack of quantitative data that directly correlates these constituents with specific bioactivities, making it difficult to ascertain their contributions to the observed effects.

Additionally, there is limited knowledge regarding the nutritional composition of the stem bark, which is crucial for assessing its potential as a nutraceutical ingredient. Finally, the antioxidant capacity of *Anthocleista djalensis* has not been systematically compared to established standards, such as ascorbic acid, which hampers the ability to evaluate its efficacy in this regard.

To address these existing gaps in knowledge, the present study aims to: (i) characterize the major phytochemical classes present in the stem bark of *Anthocleista djalensis*; (ii) provide a thorough proximate analysis of its macronutrient and mineral content; and (iii) quantify its free radical-scavenging activity using the DPPH assay, comparing the results to those of vitamin C. By integrating chemical, nutritional, and functional endpoints, this research seeks to create a comprehensive dataset that will inform the rational development of *Anthocleista djalensis*-based nutraceuticals. Additionally, this study will lay the groundwork for future mechanistic investigations into the bioactive properties of this species, ultimately contributing to a better understanding of its potential health benefits and applications in dietary supplements.

Materials and Methods

Plant Collection and Identification

The stem bark of *Anthocleista djalensis* used in this research was sourced from a farm in Benin City, Nigeria. It was identified and authenticated by a plant taxonomist from the Department of Plant Biology and Biotechnology at the Faculty of Life Sciences, University of Benin. The specimen was assigned the herbarium number UBH-A594. Following authentication, the bark was air-dried at a controlled temperature of $28 \pm 2^\circ\text{C}$ before being pulverized for further analysis.

Preparation of Plant Extract

One hundred grams of the bark powder from *Anthocleista djalensis* was extracted in 1 liter of distilled water for 24 hours, with intermittent shaking at room temperature. After the extraction period, the mixture was filtered, and the resulting filtrate was freeze-dried using a lyophilizer (Christ Alpha 1-4 LDPlus) to obtain a dry aqueous extract. The extraction yield was determined to be $11.8 \pm 0.4\%$ (w/w). For subsequent assays, stock solutions were prepared at a concentration of 10 mg/mL in water.

Phytochemical Quantification and Assay Validation

Total phenolics, flavonoids, tannins, saponins, and

alkaloids were quantified using spectrophotometric methods (Jenway 6150), following the protocols established by Singleton and Rossi (21), Harborne (22), Ilahy et al (23), Makkar et al (24), and Hagerman and Butler (25), respectively. Calibration curves, generated from eight different concentrations, demonstrated linearity with R^2 values of 0.998 for phenolics, 0.997 for flavonoids, 0.999 for tannins, 0.996 for saponins, and 0.995 for alkaloids. The limits of detection (LOD) ranged from 0.3 to $1.2\ \mu\text{g mL}^{-1}$, and the intra-assay relative standard deviations (RSDs) were less than 4%.

Nutritional Composition

The analysis of ash (26), moisture (27), crude fat (Soxhlet) (28), crude protein (micro Kjeldahl; $\text{N} \times 6.25$) (29), and crude fiber was conducted following the methods outlined by AOAC (2019). Total carbohydrate content (%) was calculated by difference, a widely accepted method when direct enzymatic hydrolysis is impractical in complex plant matrices. This approach has demonstrated a strong correlation ($r > 0.95$) with chromatographic determinations.

Measurement of DPPH Scavenging Activity

The free radical scavenging activities of each plant extract were assessed using a stable DPPH standard method, as described in references (30, 31), with minor modifications. A 0.1 mM DPPH solution was mixed with the extract at concentrations ranging from 5 to $100\ \mu\text{g mL}^{-1}$ and incubated for 30 minutes in the dark. The absorbance was measured at 518 nm, and the percentage of inhibition was plotted against the logarithm of concentration to determine the IC_{50} value.

Results

Phytochemical Screening, Nutritional Composition, and DPPH Radical Scavenging Activity of Anthocleista djalensis

Table 1 demonstrates the presence and relative abundance of various phytochemicals in the stem bark of *Anthocleista djalensis*. The analysis indicates that saponins, tannins, reducing sugars, and steroids are present in substantial quantities, as denoted by “++”. This suggests a significant potential for these compounds to contribute to the plant’s

Table 1. Phytochemicals present in Anthocleista djalensis stem bark

Constituents tested for	Inference
Saponins	++
Flavonoids	+
Tannins	++
Reducing Sugar	++
Steroids	++
Inulins	+
Alkaloids	+
Phlobatannins	+

therapeutic properties. Additionally, flavonoids, inulins, alkaloids, and phlobatannins are also present, albeit in lower quantities (indicated by “+”), which indicates their existence but in lesser abundance compared to the aforementioned compounds.

Present [Mild +] Present [Abundant ++]

Further quantitative analysis identified tannins as the most concentrated constituent, followed by phenolics, saponins, flavonoids, and alkaloids. These findings underscore the potential pharmacological significance of the stem bark, particularly in antioxidant-related applications (Table 2).

The nutritional composition derived from proximate analysis revealed that the bark contains high levels of carbohydrates and fats, along with considerable moisture and protein content. The ash content indicates a reasonable presence of minerals, while the fiber content was found to be relatively low (Table 3).

DV = Daily Value.

Both *Anthocleista djalensis* (represented in blue) and Vitamin C (represented in red) exhibited an increase in radical scavenging activity as the concentration increased. The results indicate the following:

- Vitamin C demonstrated a higher rate of inhibition compared to *Anthocleista djalensis* across all tested concentrations. This finding establishes Vitamin C as a more potent antioxidant.
- The scavenging activity of *Anthocleista djalensis* also increased significantly with concentration, suggesting that the stem bark possesses notable antioxidant properties.

While *Anthocleista djalensis* shows promise as an antioxidant source, it is important to note that its antioxidant activity is less potent than that of Vitamin C (Figure 1).

Discussion

The phytochemical analysis of *Anthocleista djalensis* stem bark (Table 1) reveals a diverse and rich profile of bioactive compounds, many of which possess well-documented pharmacological properties. Notably, saponins were identified in abundant quantities (++), consistent with

their established roles in lowering cholesterol, modulating immune responses, and exhibiting antimicrobial and anti-inflammatory effects (32). This finding supports previous research conducted by Anyanwu et al (6) and positions *A. djalensis* as a potential candidate for the management of cardiovascular and infectious diseases. Tannins, also present in high abundance, are recognized for their astringent, antioxidant, and antimicrobial properties, with applications in wound healing and gastrointestinal health. Even in moderate concentrations, these compounds contribute to the reduction of oxidative stress and the risk of chronic diseases (33, 34). Their prevalence in this species is consistent with reports from related species, such as *Anthocleista schweinfurthii*, suggesting a trend that may be characteristic of the genus. The presence of flavonoids, although moderate (+), indicates some antioxidant potential, which aligns with studies on *A. djalensis* that demonstrate variable flavonoid content depending on extraction methods and environmental conditions (35-40).

The quantitative analysis of phytochemicals in *Anthocleista djalensis* stem bark, as presented in Table 2, provides significant insights into the concentrations of various bioactive compounds. Alkaloids ($7.29 \pm 0.14\%$) are present at relatively high and consistent levels, supporting their role in the plant's analgesic, anti-inflammatory, and antimicrobial properties (41). Given their potency and narrow therapeutic index, further studies are warranted to identify specific alkaloids and assess their safety profiles.

Tannins (7111 ± 351 mg/kg) and phenolics (1607 ± 37 mg/kg) are found in substantial quantities and are likely key contributors to the extract's antioxidant and antimicrobial activities (42-44). The synergy between these compounds, particularly among condensed and hydrolysable tannins, may enhance radical scavenging capabilities (45, 46). However, potential antagonistic interactions with alkaloids could influence the overall bioactivity in vivo.

Saponins (725 ± 8.5 mg/kg) and flavonoids (336 ± 20 mg/kg)

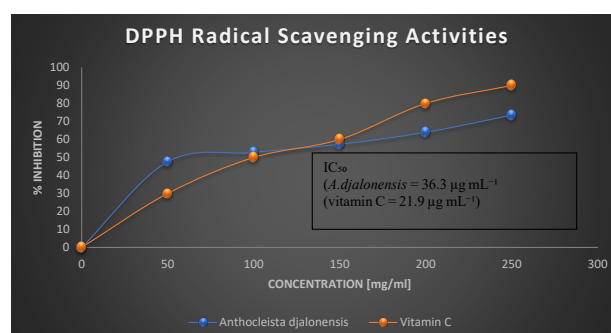


Figure 1. Graph illustrating the DPPH radical scavenging activity at various concentrations of Vitamin C and stem bark extracts from *Anthocleista djalensis*

Table 2. Phytochemicals parameters present in *Anthocleista djalensis* stem bark

S/N	Parameters	Unit	Concentration
1	Alkaloid	(%)	7.294 ± 0.14%
2	Tannins	mg/kg	7111 ± 351 mg/kg
3	Phenolics	mg/kg	1607 ± 37 mg/kg
4	Saponins	mg/kg	725 ± 8.5 mg/kg
5	Flavonoid	mg/kg	336 ± 20 mg/kg

Table 3. Proximate analysis of *Anthocleista djalensis* stem bark

<i>Anthocleista djalensis</i>	Moisture (%DV)	Protein (%DV)	Fat (%DV)	Fibre (%DV)	Ash (%DV)	Carbohydrate (%DV)
	25 ± 0.91	6.2 ± 0.052	37 ± 1.6	0.11 ± 0.010	9.3 ± 0.19	47 ± 1.8

kg) are present in moderate amounts and may contribute to immune modulation, cholesterol reduction, and antioxidant effects (47). Although these levels are lower than those found in some medicinal plants, such as *Panax ginseng*, they nonetheless enhance the plant's therapeutic profile. In comparison, alkaloid levels in *A. djalonenensis* are lower than those observed in *A. schweinfurthii* (6). However, the tannin and phenolic content is consistent with that of plants such as *Terminalia catappa*, *Cassia fistula*, and *Camellia sinensis*, which are recognized for their gastrointestinal and antioxidant benefits (24, 48, 49).

Table 3 presents the proximate composition of *Anthocleista djalonenensis* stem bark, offering essential insights into its nutritional and chemical properties. The proximate analysis encompasses moisture, protein, fat, fiber, ash, and carbohydrate content, all of which are critical for understanding the overall dietary and potential health implications of *Anthocleista djalonenensis*.

The moisture content of *Anthocleista djalonenensis* stem bark ($25 \pm 0.91\%$) is relatively high compared to many other plant materials. Elevated moisture levels can influence shelf stability, the extraction of bioactive compounds, and the overall drying processes required for utilization in traditional medicine or as dietary supplements (50). This moisture content underscores the need for appropriate drying techniques if preservation or powdering for formulation in herbal medicines is intended.

The protein content of *Anthocleista djalonenensis* ($6.2 \pm 0.052\%$) is moderate in comparison to many other plant materials. Protein is essential for plant structure and function, as well as for providing essential amino acids that may be beneficial in nutritional applications (50). Although *Anthocleista djalonenensis* has a relatively low protein content, it is unlikely to be regarded as a primary protein source. Nevertheless, its inclusion in broader food systems may contribute to overall dietary protein intake.

The fat content in *Anthocleista djalonenensis* stem bark ($37 \pm 1.6\%$) is notably high, which is atypical for many traditional medicinal plants. Elevated fat levels suggest that the plant may serve as a viable source of fatty acids, which are associated with various health benefits, including anti-inflammatory properties and support for cardiovascular health (51). However, this high fat content also necessitates careful consideration of lipid-based extraction methods if bioactive compounds are to be isolated for pharmacological studies.

The fiber content in *Anthocleista djalonenensis* stem bark ($0.11 \pm 0.010\%$) is extremely low, which may limit its utility in promoting digestive health. Dietary fiber plays a crucial role in gut health by aiding digestion and preventing constipation (51). This low fiber level may influence recommendations for its use in food matrices, particularly in contexts where fiber content is typically desired for associated health benefits.

A lipid-rich matrix can enhance the oral bioavailability of lipophilic phytochemicals, such as sterols and triterpenoids (52). However, this high fat content

also presents challenges regarding oxidative stability, requiring the incorporation of antioxidant co-formulants or encapsulation methods to prevent rancidity (53). Additionally, the low dietary fiber content limits the prebiotic benefits typically sought in functional foods (54). To address this, blending the bark powder with high-fiber carriers, such as oat bran, could help balance macronutrient profiles while preserving bioactive compounds (55). Furthermore, the elevated fat content may increase energy density, an important consideration for products aimed at weight management (56).

The ash content of *Anthocleista djalonenensis* ($9.3 \pm 0.19\%$) provides an estimation of the mineral content within the plant material. An ash content of approximately 9.3% indicates that *Anthocleista djalonenensis* contains a variety of minerals that are essential for various physiological functions when consumed (57). This level of mineral content can enhance the nutritional profile of traditional recipes or formulations that incorporate this species, potentially contributing to overall health and well-being.

The carbohydrate content of *Anthocleista djalonenensis* ($47 \pm 1.8\%$) is significantly high, constituting the bulk of its composition. Carbohydrates serve as a primary energy source and are essential for maintaining energy levels in various biological processes (58). This high carbohydrate content suggests that the stem bark could be a valuable energy source in traditional diets or natural remedies.

The DPPH assay is a widely used method for evaluating the antioxidant capacity of various substances. It measures the ability of antioxidants to donate an electron or hydrogen atom to the DPPH radical, which leads to the neutralization of the radical and a subsequent reduction in its absorbance. A decrease in absorbance correlates with an increase in radical scavenging activity, indicating higher antioxidant potential (59).

Antioxidant Activity

As illustrated in Figure 1, Vitamin C—commonly used as a reference standard—exhibits strong DPPH radical scavenging activity that increases with concentration, eventually plateauing at higher doses, which indicates saturation. Similarly, the extract of *Anthocleista djalonenensis* stem bark shows a concentration-dependent increase in scavenging activity. At lower concentrations (e.g., 5 mg/mL), its antioxidant activity is significantly lower than that of Vitamin C; however, as the concentration increases, the efficacy of the extract improves, approaching that of Vitamin C at higher levels. This trend suggests that the extract may contain multiple antioxidant constituents that could act synergistically to enhance its radical-scavenging capacity.

While Vitamin C consistently outperforms the extract at lower concentrations, the extract's performance at higher concentrations indicates promising antioxidant potential. These findings highlight the need for further investigation into optimizing extraction methods and formulation strategies to maximize the radical-scavenging properties

of *Anthocleista djalonenensis* extract. Such enhancements could expand its applications in functional foods or dietary supplements aimed at combating oxidative stress and promoting health (55).

The demonstrated antioxidant activity of *Anthocleista djalonenensis* highlights its potential for developing functional foods and nutraceuticals. Antioxidants are essential in mitigating oxidative stress-related diseases, providing protective effects against various conditions, including heart disease, cancer, and neurodegenerative disorders (60).

Given its traditional uses and substantiated antioxidant capacity, *Anthocleista djalonenensis* presents a valuable opportunity for integration into contemporary medicinal practices. Its rich history in traditional medicine, combined with scientific evidence supporting its antioxidant properties, positions it well for inclusion in formulations designed to enhance overall health and combat oxidative stress.

Clinical and Nutraceutical Relevance

The bioactive profile of *Anthocleista djalonenensis* bark reveals several promising clinical and nutraceutical opportunities. The abundance of tannins in the extract is noteworthy, as these compounds are recognized for their astringent and antimicrobial properties. Tannins can support wound healing when applied topically and may also be formulated into oral supplements aimed at addressing gastrointestinal conditions, potentially aiding in the management of issues such as diarrhea and inflammation.

Additionally, the presence of phenolic compounds in *Anthocleista djalonenensis* is significant due to their established antioxidative mechanisms. These compounds are valuable in combating oxidative stress, which is associated with aging, cardiovascular disease, and metabolic syndrome.

The alkaloid content of *Anthocleista djalonenensis* reflects both a high concentration and low variability, indicating consistent accumulation across samples and analytical replicability. This consistency reinforces the therapeutic relevance of alkaloid-rich fractions, many of which are recognized for their antimicrobial, anti-inflammatory, and neuropharmacological effects. However, due to the potent bioactivity and narrow therapeutic windows often associated with alkaloid compounds, it is crucial to further characterize specific alkaloid subtypes and conduct detailed toxicological profiling prior to clinical application.

These observations highlight the necessity of conducting in vivo studies to evaluate systemic bioavailability, pharmacodynamics, and safety under physiological conditions. Such studies are essential not only for validating the antioxidant and therapeutic claims derived from in vitro assays but also for clarifying potential synergistic or antagonistic interactions among the various constituent classes present in the extract.

Collectively, the phytochemical richness of *Anthocleista*

djalonenensis bark—marked by the presence of abundant tannins, phenolics, and alkaloids—alongside its confirmed free radical scavenging capacity, positions it as a strong candidate for nutraceutical or phytopharmaceutical development. These bioactive compounds not only contribute to the antioxidant properties of the bark but also suggest potential therapeutic applications in various health-related contexts. However, successful formulation of products derived from *Anthocleista djalonenensis* will necessitate addressing certain challenges, particularly its high fat and low fiber content. These factors can impact product stability, efficacy, and consumer acceptability.

Conclusion

The study confirmed that *Anthocleista djalonenensis* stem bark is rich in bioactive compounds, particularly tannins and phenolics, which contribute significantly to its therapeutic and nutritional potential. The plant's notable antioxidant properties not only support its traditional medicinal applications but also highlight its potential for development into nutraceuticals and functional foods. Additionally, the high carbohydrate and fat content further enhance its utility as a nutritional supplement, making it a versatile candidate for various health-related formulations. Given its broad range of bioactive compounds, *Anthocleista djalonenensis* holds promise for both pharmaceutical and industrial applications. Future research should prioritize isolating specific compounds and understanding their mechanisms of action to elucidate how they contribute to the plant's health benefits.

Authors' Contribution

Conceptualization: Joseph Raymond Enoghase, Silvanus Olu Innih.

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Writing—review & editing: Joseph Raymond Enoghase, Silvanus Olu Innih.

Competing Interests

The authors declare that there are no known conflicts of interest or personal relationships that could have influenced the work reported in this paper.

Declaration of Data Availability

The data supporting the findings of this study can be obtained from the corresponding author upon reasonable request. Interested parties can contact the corresponding author directly via their email address for further information.

Ethical Approval

The research adhered to the guidelines set forth by the research ethics committee for animal handling and treatment at the University of Benin's College of Medical Sciences. Ethical approval

for the study was granted, with the assigned approval number CMS/REC/2023/340.

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