

Microscopic studies, phytochemical, mineral composition, and antioxidant activity of the stem bark of *Ganophyllum giganteum* (A. Chev.) Hauman (Sapindaceae) from the Ebola River Basin, Nord Ubangi, Democratic Republic of the Congo

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ARTICLE INFO

Received: 07 August 2024

Accepted: 19 September 2024

Published: 03 October 2024

Keywords:

Ganophyllum giganteum, histological examination, phytochemical screening, elemental analysis, antioxidant efficacy

Peer-Review: Externally peer-reviewed

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To cite:

Lengbiye, E. M., Ngbolua, K. N., Balela, W. B., Bekomo, J. I., Tshibangu, D. S., Mbing, J. N., Pegnyemb, D. E., & Mpiana, P. T. (2024).

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Hauman (Sapindaceae) from the Ebola River Basin, Nord Ubangi, Democratic Republic of the Congo. *Orapuh Journal*, 5(5), e1145

<https://dx.doi.org/10.4314/orapj.v5i5.45>

ISSN: 2644-3740

Published by *Orapuh, Inc.* (info@orapuh.org)

Editor-in-Chief: Prof. V. E. Adamu

Orapuh, Inc., UMTG PMB 405, Serrekunda, The

Gambia, editor@orapuh.org.

ABSTRACT

Introduction

Traditional uses of *Ganophyllum giganteum* (A. Chev.) Hauman and previous studies suggest that this plant may contain secondary metabolites with medicinal properties. The plant has been historically utilized for various therapeutic purposes, yet scientific exploration of its chemical and biological characteristics remains limited.

Purpose

This study aims to identify the secondary metabolites present in the stem bark of *Ganophyllum giganteum* (A. Chev.) Hauman, examine its histological elements, determine its mineral composition, and assess its antioxidant activity.

Methods

The study employed microscopic examination to identify histological features, phytochemical analysis to characterize secondary metabolites, and mineral analysis using a PHYWE 4.0 X-ray diffractometer. Antioxidant activity was assessed using ABTS^{•+} and DPPH assays, with both aqueous and ethanolic extracts of the plant tested.

Results

Microscopic analysis revealed histological elements such as fibre fragments and palisading parenchyma in the stem bark. The phytochemical profile identified a variety of secondary metabolites, including polyphenols, anthocyanins, leuco-anthocyanins, flavonoids, alkaloids, quinones, tannins, saponins, triterpenes, and steroids. The antioxidant activity results showed that both aqueous and ethanolic extracts exhibited significant antioxidant power, with the ethanolic extract demonstrating the highest activity (IC₅₀ = 0.71±0.00 µg/mL) compared to the aqueous extract (IC₅₀ = 15.93±2.57 µg/mL) and the control (quercetin, IC₅₀ = 3.21±0.99 µg/mL). The mineral composition analysis indicated the presence of essential elements such as calcium, potassium, phosphorus, selenium, iron, vanadium, manganese, copper, zinc, silica, sulphur, cobalt, and cadmium.

Conclusion

The study provides scientific evidence validating the traditional use of *Ganophyllum giganteum* (A. Chev.) Hauman for medicinal purposes. The plant's rich chemical composition and notable antioxidant and antimicrobial activities highlight its potential as a source for developing drugs and nutraceuticals. These findings underscore the plant's potential role in managing diseases and contribute to future research in the development of therapeutic agents based on this plant.

INTRODUCTION

Antibiotic therapy is a crucial tool in combating microbial infections; however, the growing problem of antibiotic resistance poses significant challenges to controlling the spread of resistant microorganisms (Bishnu et al., 2020; Kevin et al., 2024). This resistance is largely driven by the extensive use of antibiotics in both humans and animals. Infections caused by pathogens also lead to the production of free radicals in the body, triggering inflammatory responses and contributing to the onset of diseases such as cancer, cardiovascular conditions, and degenerative disorders (Zeghal & Sahnoun, 2013; Syed et al., 2023).

Given these challenges, the study of medicinal plants, long used in traditional medicine, becomes even more critical. These plants are a valuable source of secondary metabolites such as polyphenols, flavonoids, alkaloids, saponins, terpenoids, quinones, and tannins, which offer diverse pharmacological benefits (El-Shahir et al., 2022). In addition, plants containing essential minerals like selenium, iron, potassium, and sodium play a vital role in maintaining bodily functions, including muscle contraction, metabolism, and water balance (Lengbiye et al., 2020).

Among these medicinal plants, *Ganophyllum giganteum* (A. Chev.) Hauman, a tree native to the Democratic Republic of the Congo, stands out for its traditional therapeutic uses. Its bark maceration and decoction have been employed to treat respiratory and digestive illnesses, epilepsy, dysmenorrhea, sterility, trypanosomiasis, and wounds. Previous studies have reported cytotoxic activity in its leaf extracts and identified several bioactive compounds, including zanhic acid, medicagenic acid, and acylated oleane-type saponins, which display anti-inflammatory and cytotoxic properties (Lamidi et al., 2005; Elier et al., 2013).

Despite its widespread use in traditional medicine, scientific knowledge of the phytochemical composition, mineral content, and biological activities of *Ganophyllum giganteum*, particularly its antioxidant properties, remains limited. This gap underscores the need for comprehensive studies to validate the traditional uses of this plant and explore its potential for inclusion in modern pharmacopoeia. The present study aims to address this

gap by investigating the histological characteristics, phytochemical profile, mineral composition, and antioxidant activity of extracts from the stem bark of *G. giganteum* (A. Chev.) Hauman. The findings could not only validate its traditional therapeutic applications but also contribute to the discovery of new natural compounds for treating infectious and free radical-related diseases.

METHODS

Plant Material

The plant species for this study was selected based on prior ethnobotanical surveys. The stem bark of *Ganophyllum giganteum* (A. Chev.) Hauman was collected from Yakoma in the Nord-Ubangi Province of the Democratic Republic of the Congo (DRC), located at approximately 22° 35' and 23° 37' longitude East, latitude 3° 48' and 4° 6' North, and an altitude of 470 m above sea level (Ngunde-te-Ngunde et al., 2020). The species was identified (No. 2127) at the Institut National d'Études et de Recherche Agronomique, Faculty of Science and Technology, University of Kinshasa. Subsequently, the stem bark was air-dried at room temperature (approximately 27 °C) for two weeks in the Laboratory of Ethnobiology and Medical Phytochemistry and then finely powdered for further analyses.

Figure 1: Geographical distribution of *Ganophyllum giganteum* (A. Chev.) Hauman (Source: <https://powo.science.keew.org/>)



Antioxidant Activity

Antioxidant activity was assessed using aqueous and ethanolic extracts, following the ABTS and DPPH assay methods as described by Kapepula et al. (2016) and Lengbiye et al. (2020).

a) Radical Scavenging Capacity of DPPH

The DPPH method is commonly used to evaluate antioxidant activity based on the degradation of the DPPH radical (2,2-Diphenyl-1-Picrylhydrazyl), a purple-colored free radical. When an antioxidant is added, it reduces the radical by donating an electron or hydrogen atom, resulting in discoloration, which is proportional to the antioxidant concentration and measured spectrophotometrically at 517 nm.

For this test, a DPPH solution was prepared by dissolving 3.2 mg of DPPH in 100 mL of 80% methanol and kept in the dark for at least one hour. The absorbance of the solution was adjusted between 0.7 and 0.05 with methanol. A control was prepared by mixing 20 μ L of methanol with 1980 μ L of the DPPH solution. For the samples, 20 μ L of each sample was mixed with 1980 μ L of DPPH solution and incubated in the dark for 30 minutes. The absorbance of the solutions (control, blank, and samples) was measured at 517 nm. The decrease in absorbance compared to the control indicates antioxidant activity, with a greater reduction in absorbance reflecting higher activity.

The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\% \text{ Inhibition} = [1 - (A_x/A_c)] \times 100$$

Where:

A_x = Absorbance of the DPPH radical in the presence of the extract

A_c = Absorbance of DPPH (control solution).

The DPPH trapping activity of extracts was expressed as IC₅₀. Different IC₅₀ values were determined using [GraphPad Prism version 6.0 software](#). Each sample was measured in triplicate.

b) ABTS Radical Scavenging Capacity

The ABTS assay involves the reaction of ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) with potassium persulfate ($K_2S_2O_8$) or sodium persulfate, forming a blue-green ABTS cationic radical. When an antioxidant is added, it reduces the radical, causing discoloration, which is proportional to the antioxidant concentration and measured at 734 nm.

To prepare the reagents, 20 millimoles of ABTS were dissolved in 500 μ L of distilled water (solution A), and 10 millimoles of potassium persulfate in 500 μ L of distilled water (solution B). Equal volumes of solutions A and B were mixed and kept in the dark for 12-16 hours to generate the ABTS radical stock solution. The stock solution was diluted with methanol to achieve an absorbance between 0.800 and 1.000. For the assay, 20 μ L of methanol was mixed with 1980 μ L of the ABTS solution in a test tube (control). In separate tubes, 20 μ L of the sample solution was added to 1980 μ L of ABTS radical solution, incubated in the dark for 30 minutes, and the absorbance of the blank (methanol), control, and samples was measured at 734 nm.

The percentage inhibition of the ABTS radical was determined using the following formula:

$$\% \text{ Inhibition} = [1 - (A_x/A_c)] \times 100$$

Where:

A_x = Absorbance of the ABTS radical in the presence of the extract

A_c = Absorbance of the ABTS (control solution).

The ABTS trapping activity of extracts was expressed as IC₅₀. Different IC₅₀ values were determined using [GraphPad Prism version 6.0 software](#). Each sample was measured in triplicate.

Microscopic Examination of the Powder

Powder micrography is one of the fundamental quality control methods for herbal medicines. It is crucial to prepare a microscopic sample for observation, distinguishing the different components of the plant powder.

Reagents Used

1. Lactic acid reagent or Steimetz reagent, which highlights starch (intense violet staining) and lignified elements like sclerotic cells and vessels (intense yellow staining).
2. 20% KOH, which destroys starch, making other elements more visible.
3. Water, to observe starch grains in more detail.

Procedure

Using a Pasteur pipette, place a few drops of distilled water on a slide, add a small quantity of pulverized powder, and cover with a coverslip. Observe the starch grains in the organs (stem bark) under a microscope. Similarly, add lactic acid reagent or Steimetz reagent instead of water to highlight various histological structures, then observe under the microscope (Lengbiye et al., 2020).

Phytochemical Analysis

Phytochemical screening was carried out on aqueous and organic extracts to identify chemical groups, following the standard protocol described by Bruneton (1999) and Tiwari et al. (2011).

Preparation of Aqueous Extracts

Five grams of *Ganophyllum giganteum* (A. Chev.) Hauman stem bark powder was mixed with 50 mL of distilled water and allowed to stand for 24 hours before filtering. The same procedure was followed for the organic extract, which stood for 48 hours in an organic solvent (e.g., methanol, ethanol, dichloromethane). The filtrate was the macerate.

Testing the Aqueous Phase

1. **Polyphenol Analysis**
 - *Reagents:* Burton's reagent (FeCl_3 2%), $\text{K}_3\text{Fe}(\text{CN})_6$ 1% (1:1 v/v).
 - *Procedure:* Add 1 mL of aqueous extract to a test tube and introduce a few drops of Burton's reagent using a Pasteur pipette. A deep blue colour in the solution, potentially accompanied by a precipitate, confirms the presence of polyphenols, including flavonoids, quinones, anthocyanins, tannins, and leuco-anthocyanins, which can be tested further (Bruneton, 1999).

2. **Testing for Flavonoids, Anthocyanins, Leuco-anthocyanins, Tannins, Alkaloids, Saponins, and Bound Quinones**

- Reagents and procedures followed standard phytochemical testing techniques as described by Tiwari et al. (2011).

Determination of Mineral Content

The mineral content of the samples was determined using a PHYWE 4.0 X-ray diffractometer equipped with a tungsten target. The diffraction patterns were recorded over a 2θ range from 20° to 90° , enabling comprehensive analysis of the mineral composition. This technique allowed for precise identification and quantification of various minerals present in the sample. The use of tungsten as the X-ray source provided high resolution and sensitivity in detecting trace elements, making it suitable for detailed mineral analysis (Carlos et al., 2023).

RESULTS AND DISCUSSION

Antioxidant Activity

Table 1:

Antioxidant activity results of *Ganophyllum giganteum* (A. Chev.) Hauman, stem bark

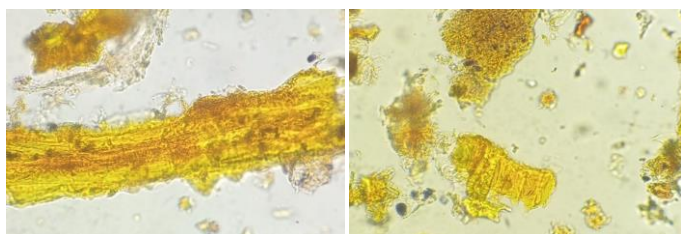
Extracts	<i>Ganophyllum giganteum</i> (A. Chev.) Hauman, stem bark (IC ₅₀ in µg/mL).	
	ABTS ^{•+}	DPPH [•]
Ethanollic	0.71±0.00	24.20±3.54
Aqueous extract	15.93±2.57	21.11±2.31
Quercétine	3.21±0.99	

The antioxidant activity of *Ganophyllum giganteum* (A. Chev.) Hauman stem bark extracts was determined using the ABTS^{•+} and DPPH[•] assays (Table 1). The results indicated that both the aqueous and ethanolic extracts of *Ganophyllum giganteum* (A. Chev.) Hauman exhibited antioxidant properties and were capable of inhibiting free radicals. The ethanolic extract demonstrated the highest antioxidant activity in the ABTS^{•+} assay with an IC₅₀ value of 0.71 ± 0.00 , followed by the aqueous extract, which had an IC₅₀ value of 15.93 ± 2.57 . Similarly, both extracts exhibited strong antioxidant activity in the DPPH[•] test. These findings suggest that the plant possesses significant antioxidant properties and may have potential in managing free radicals and related diseases. Antioxidant molecules, such as ascorbic acid, α -tocopherol, flavonoids, and tannins, are known to neutralise free

radicals like DPPH and ABTS due to their hydrogen-donating capacity (Talbi et al., 2015). The presence of secondary metabolites, such as polyphenols, flavonoids, and tannins, in the plant extracts is likely responsible for the observed antioxidant activity (Bongo et al., 2017). This supports the traditional use of this plant in managing diseases linked to free radicals, including cancers, degenerative diseases, cardiovascular, and inflammatory diseases (Syed et al., 2023).

Microscopic Studies

Figures 2: A) Fiber fragment and B) Palissadic parenchyma



Microscopic examination of *Ganophyllum giganteum* (A. Chev.) Hauman stem bark revealed several histological features, including fibre fragments and palisade parenchyma (Figures 2: A & B). These histological elements are characteristic of the plant species. Given its use in traditional medicine, it is crucial to identify the histological characteristics of the plant to standardise the drugs derived from it. Moreover, this analysis can help detect anti-nutritional factors such as oxalate crystals, which may lead to kidney disease if consumed in excess (Lengbiye et al., 2020).

Phytochemical Study

Table 2: Phytochemical screening results

Compounds	Stem bark of <i>Ganophyllum giganteum</i> (A. Chev.) Hauman
Polyphenols	+
Flavonoïds	+
Anthocyanins	+
Leuco-anthocyanins	+
Tannins	+
Quinones	+
Alkaloids	+
Saponins	+
Tri-terpenes and Steroids	+

The phytochemical screening of *Ganophyllum giganteum* (A. Chev.) Hauman stem bark revealed the presence of secondary metabolites of biological interest, such as anthocyanins, flavonoids, alkaloids, polyphenols, and

triterpenes, corroborating the findings of Lamidi et al. (2005). Similarly, the presence of saponins in the extracts was confirmed, as reported by Galarraga et al. (2014) (Table 2).

Mineral Compounds

Table 3: Mineral composition of the stem bark of *Ganophyllum giganteum*

Energy (Kev)	Impulsions (imp/s)	FWHM (nm)	Peak assignment
1.414	3.425	0.013	Se-La
1.715	2.000	0.013	Si-Ka
2.137	1.967	0.016	P-Kb
2.325	2.024	0.007	S-Ka
3.054	3.063	0.011	Cd-La
3.528	10.812	0.016	K-Kb
3.730	15.700	0.010	Ca-Ka
5.032	1.515	0.006	V-Ka
5.759	7.117	0.017	Mn-Ka
6.548	6.687	0.009	Fe-Ka
7.016	4.676	0.009	Co-Ka;
8.351	0.799	0.013	Cu-Ka
8.589	0.944	0.014	Zn-Ka

Figure 3: Mineral composition spectrum of *Ganophyllum giganteum* (A. Chev.) Hauman stem bark

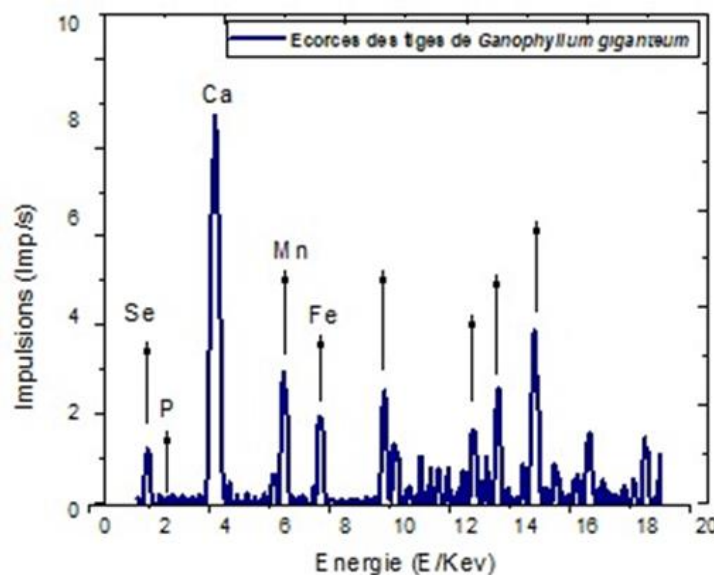


Table 3 and Figure 3 illustrate the mineral composition of the stem bark of *Ganophyllum giganteum* (A. Chev.) Hauman, showing the presence of 13 mineral elements. Macro-elements such as calcium, potassium, and

phosphorus were detected, alongside 10 microelements, including selenium, iron, vanadium, manganese, copper, zinc, silica, sulphur, cobalt, and cadmium. Selenium is known for its role in plant defence mechanisms against pathogens and herbivores due to the production of volatile selenoid compounds, which are toxic to these organisms (John et al., 2006). The accumulation of these mineral elements in plants is influenced by ecological factors (Kitadi et al., 2020).

Minerals, as Soetan et al. (2010) pointed out, are essential inorganic nutrients required in small amounts to support various biochemical reactions. Elements such as copper, selenium, zinc, iron, and manganese act as cofactors for enzymes essential for physiological processes, while sodium and potassium maintain osmotic balance. Cobalt is vital for the synthesis of vitamin B12 and its role in DNA biosynthesis and amino acid metabolism (Arinola et al., 2008). Selenium, an essential mineral, also plays a significant role as an antioxidant and immunomodulator, surpassing vitamins E, C, A, and beta-carotene in potency. However, excessive intake of certain minerals can disrupt homeostasis, leading to toxic effects (Ngbolua et al., 2019; Lengbiye et al., 2020). Justyna et al. (2014) identified 22 elements essential for animal growth and health, including macro-elements and microelements crucial for structural, physiological, catalytic, and regulatory functions. Mineral deficiencies, due to poor dietary intake, inadequate absorption, or increased demand, can impair growth, nutrient absorption, and immunity.

The presence of cadmium in *Ganophyllum giganteum* poses potential health risks, such as kidney disease, lung damage, and bone fragility. Ecological factors are known to influence mineral accumulation in plants (Kitadi et al., 2020).

Implications for Medicinal Plant Research

This study contributes significantly to medicinal plant research, especially in promoting African plants. The identification of bioactive compounds, such as flavonoids, tannins, and saponins, suggests that *Ganophyllum giganteum* has notable therapeutic potential. These compounds exhibit antioxidant, anti-inflammatory, and antimicrobial properties, which may justify the plant's traditional use in treating inflammatory and infectious

diseases. From a public health standpoint, the antioxidant activities of *G. giganteum* indicate its potential preventive role against oxidative stress-related diseases, such as cardiovascular diseases, diabetes, and certain cancers. Additionally, the presence of essential minerals like calcium, magnesium, and potassium adds a nutritional dimension to this plant, which could aid in addressing mineral deficiencies in vulnerable populations.

Study Limitations

Despite the valuable insights gained, several limitations warrant further investigation. The in vitro antioxidant activity observed in this study provides preliminary evidence of the plant's potential benefits but does not necessarily reflect its efficacy in vivo. Additional in vivo studies are necessary to confirm the therapeutic potential of *Ganophyllum giganteum*. These studies would validate the observed antioxidant effects and assess the plant's overall safety and efficacy in physiological conditions. Furthermore, exploring the bioavailability and metabolism of the plant's compounds in vivo will provide a more comprehensive understanding of its potential benefits and risks. Addressing these limitations will strengthen the evidence supporting the plant's use in both traditional and modern medicine.

CONCLUSION AND RECOMMENDATIONS

This study assessed the histological, chemical, and mineral composition of *Ganophyllum giganteum* (A. Chev.) Hauman stem bark. Histological analysis revealed the presence of fibre fragments and palisade parenchyma. The phytochemical profile of the stem bark was dominated by secondary metabolites, including polyphenols, anthocyanins, leuco-anthocyanins, flavonoids, alkaloids, quinones, tannins, saponins, triterpenes, and steroids. The mineral composition included essential elements such as calcium, potassium, phosphorus, and iron, but also detected cadmium, a heavy metal with known toxic effects. Antioxidant activity tests indicated that the plant exhibits significant antioxidant properties.

The findings support the traditional use of *G. giganteum* in treating various illnesses in the Democratic Republic of the Congo, highlighting its potential as a source of beneficial secondary metabolites and essential minerals. Regular consumption of medicinal plants with antioxidant, anti-

inflammatory, and antimicrobial properties is generally recommended for health support. However, given the presence of cadmium, it is crucial to approach its use with caution.

To ensure the plant's safety and efficacy, extensive in vivo studies are necessary. These studies should validate the observed in vitro antioxidant effects, assess the physiological benefits, and investigate potential toxicities associated with cadmium. Further research should focus on isolating and characterising beneficial compounds from the plant while evaluating their safety under realistic physiological conditions to confirm their therapeutic potential. Additionally, it would be valuable to explore the plant's antimicrobial activity in greater detail and assess the bioavailability of its active compounds in vivo.

Acknowledgements: The authors express their gratitude to the University of Kinshasa, Democratic Republic of the Congo, for the technical support received.

Ethics Approval: Ethical approval was obtained from the Life Sciences Department, Faculty of Science and Technology (University of Kinshasa), under protocol number 024/CDB/MSV/FST/UNIKIN.

Conflicts of Interest: None declared.

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