Research Article
Sciences of Phytochemistry

In Vitro Antioxidant, Antimicrobial and Anti-helminthic Activity of Methanolic Extract of *Aganope Leucobotrya*

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[The author informations are in the declarations section. This article is published by ETFLIN in Sciences of Phytochemistry, Volume 5, Issue 2, 2026, Page 329-335. DOI: 10.58920/sciphy0502645]

Received
02 April 2026

Revised
22 May 2026

Accepted
12 September 2026

Published
01 November 2026

Editor
Bharath Kumar Chagaleti

Keywords: Aganope leucobotrya, phytochemical screening, antioxidant activity, anthelmintic activity, antimicrobial activity, plant extracts.

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Abstract: The stem bark of *Aganope leucobotrya* is effective for diarrhea, as a blood tonic, for bone healing, and nose bleeding. The purpose of this work was to screen for the phytoconstituents and evaluate the antimicrobial, anthelmintic, and antioxidant activities of the methanolic extract of the stem bark of *A. leucobotrya*. Standard methods were employed for phytochemical screening. The broth dilution method was utilized in assessing antimicrobial activity. 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay and hydrogen peroxide scavenging assay were employed to assess antioxidant activity. *In vitro* anthelmintic activity was determined using earthworms (*Eudrilus eugeniae*). Phenols, tannins, saponins, quinones, and polyphenols were identified to be present in the extract. A minimum inhibitory concentration (MIC) of 12.5 ± 0.01 mg/mL was obtained as the highest activity for the methanol extract against *Streptococcus pyogenes* and *Candida albicans*. The half-maximal inhibitory concentration (IC50) of the methanol extract and the reference drug for the DPPH scavenging activity were 49.96 ± 0.10 µg/mL and 32.35 ± 0.05 µg/mL, respectively. At 20 mg/mL, the paralysis and death times for both the methanol extract and Mebendazole drug were 15 min, 36 min, and 29 min, 50 min, respectively. The IC50 obtained for the hydrogen peroxide scavenging for the methanol extract and gallic acid were 185.63 ± 0.152 µg/mL and 151.80 ± 0.094 µg/mL, respectively. The methanol extract of *A. leucobotrya* possesses antioxidant, anthelmintic, and antimicrobial activities.

Introduction

Herbal plants have long been used to treat various illnesses and health conditions (1). Many plants naturally produce bioactive compounds to defend against diseases, pests, fungi, and herbivores. Given the complexity of phytochemicals within a single plant, the effectiveness of using the whole plant for medicinal purposes remains a subject of debate. However, these plants serve as natural sources of pharmacologically active compounds, forming the basis of modern medicine (15). Medicinal plants continue to attract significant interest due to their accessibility, affordability, and widespread use in traditional healing practices (5). Phytochemicals can be derived from various plant parts, including roots, stem bark, leaves, fruits, flowers, and seeds (13). Studies have shown that numerous bioactive plant compounds exhibit antibacterial, antioxidant, and anti-inflammatory properties (4). Many of these compounds serve as the foundation for antioxidants, antimicrobial agents, anthelminthics, anti-inflammatory drugs, and anticancer treatments either in their natural state or as synthetic or semi-synthetic derivatives (19). The biological activity of medicinal plants is primarily attributed to these phytochemicals (3). Antibiotic resistance remains a growing global concern, as microbial adaptation continues to undermine the efficacy of many antibiotics. This resistance has significantly compromised the treatment and prevention of various infectious diseases (16). Additionally, oxidative stress caused by an overproduction of reactive oxygen and nitrogen species (RONS) leads to cellular damage and is linked to aging, cancer, atherosclerosis, liver cirrhosis, and neurodegenerative diseases like Alzheimer's (14). Antioxidants play a crucial role in neutralizing oxidative stress, making them essential in disease prevention and therapy (7). *Aganope leucobotrya* is a leguminous climbing shrub native to Sierra Leone, southern Nigeria, Ghana, and the Ivory Coast. It is characterized by pink and white flowers and has been

traditionally used in Ghana to treat diarrhea, as a blood tonic, to promote bone healing, stop nosebleeds, and combat bacterial infections. Since its discovery in 1987, certain species of *Aganope* have been studied for their antibacterial, anti-inflammatory, and anthelmintic properties. However, scientific research on *A. leucobotrya* remains limited. A closely related species, *Derris elliptica*, has been extensively studied and is widely used in East Asia as an insecticide and preservative. Thin-layer chromatography analysis of *D. elliptica* has revealed high concentrations of isoflavonoids and rotenoids in its stem and root, which contribute to its anti-inflammatory and antibacterial properties. Studies have confirmed its effectiveness against *Bacillus subtilis*, *Staphylococcus aureus* (which causes skin infections), and *Escherichia coli* (a common foodborne pathogen). Additionally, research has validated its anthelminthic properties. Despite its traditional medicinal use, no scientific studies have been conducted to evaluate the antimicrobial, antioxidant, and anthelminthic properties of *A. leucobotrya*. This study seeks to fill that gap by investigating its phytochemical composition and potential pharmacological applications.

Materials and Methods

Chemicals and Reagents

Except for the standard drugs, all chemicals were obtained from Sigma-Aldrich Co. Ltd. (Irvine, UK), while analytical-grade organic solvents were procured from BDH Laboratory Supplies (England).

Sample Collection and Identification

Stems of *A. leucobotrya* were collected in December 2022 from Aburi, Yaw Duodu, in the Akuapim South District of the Eastern Region of Ghana (latitude: 6°37′44.96″N; longitude: 0°43′21″W), with the assistance of a local herbalist. The plant was identified and authenticated by Mr. Clifford Asare of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. A voucher specimen (KNUST/HMI/2022/SB080) was deposited for future reference. The stems were washed, cut into smaller pieces, and air-dried at room temperature for 21 days until a constant weight was achieved. The dried material was pulverized into a fine powder and stored in airtight containers. Extraction was carried out by cold maceration using 97% methanol. The extract was concentrated under reduced pressure using a rotary evaporator (Cole-Parmer N-1110, China), further dried to remove residual solvent, and stored under refrigeration until use.

Phytochemical Screening

The pulverized sample and crude extract were subjected to qualitative phytochemical screening using standard methods as previously described (5).

In Vitro Antioxidant Activity

DPPH Radical Scavenging Assay

Extracts was prepared at concentrations of 20, 40, 60, 80, and 100 µg/mL. Ascorbic acid served as the reference standard, while distilled water was used as the negative control. In a 96-well microtiter plate, 150 µL of DPPH solution was mixed with 50 µL of each sample. The mixtures were incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a microplate reader. Antioxidant activity was expressed as IC_{50} values.

Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging activity was determined using a modified method described in (5), based on the formation of a red-orange tris (1, 10-phenanthroline)-iron (II) complex. A stock solution of the extract (1000 µg/mL) was prepared and diluted to concentrations of 200, 400, 600, and 800 µg/mL. Gallic acid was used as the reference standard. To each test tube, 0.5 mL of ferric ammonium sulfate (1 mM) and 3 mL of sample solution were added, followed by 0.13 mL of hydrogen peroxide (5 mM). The mixture was incubated in the dark for 5 min. Subsequently, 3 mL of 1, 10-phenanthroline (1 mM) was added, mixed thoroughly, and allowed to stand for 10 min at room temperature. Absorbance was measured at 510 nm. The negative control contained ferric ammonium sulfate, distilled water, and 1, 10-phenanthroline without extract.

In Vitro Anthelmintic Assay

Worm Collection and Authentication

Adult earthworms (*Eudrilus eugeniae*) were collected from a moist area behind the Department of Theoretical and Applied Biology, KNUST, Kumasi, Ghana. The organisms were authenticated by Mr. Lawrence Yeboah of the same department.

Anthelmintic Activity

The assay was conducted with slight modifications to the method described in (2). The extract was prepared at concentrations of 1.25, 2.50, 5.00, 10.00, and 20.00 mg/mL. Mebendazole served as the reference drug, while distilled water was used as the negative control. Four worms of similar size were placed in Petri dishes containing 50 mL of each test solution. Time to paralysis (no movement upon shaking) and death (no movement even after immersion in warm water at 50 °C) were recorded. All experiments were conducted in triplicate, and results were expressed as mean ± SEM.

Antimicrobial Assays

Test Microorganisms

The antibacterial activity of the extract was evaluated against *Candida albicans, Escherichia coli, Salmonella typhi, Streptococcus pyogenes,* and *Staphylococcus aureus*. The organisms were obtained from the Pharmaceutical Microbiology Unit, KNUST, Kumasi, Ghana, and cultured in nutrient broth at 37 °C for 18 h (23).

Preparation of Microbial Suspensions

Microbial suspensions were prepared by diluting cultures in sterile saline to match the 0.5 McFarland standard (≈1.5 × 10^8 CFU/mL).

Broth Microdilution Method (MIC Determination)

A modified broth microdilution method was used [5]. Briefly, 100 µL of double-strength nutrient broth was added to each well of a 96-well plate. Extracts were added to achieve concentrations of 50, 25, 12.5, 6.25, 3.125, and

Table 1. % yield of methanolic extract.

Mass of pulverized sample	Mass of extract	% yield
1000 g	100 g	10.0 %

Table 2. Phytoconstituents in the pulverized sample and the methanol extract.

Secondary metabolites	Pulverized sample	Extract
Alkaloids	-	-
Phenols	+	+
phytosterol	-	-
Saponins	+	+
Steroids	-	-
Tannins	+	+
Terpenoids	-	-
Flavonoids	-	-
Quinones	+	+
Polyphenols	+	+
Carotenoids	-	-
Note: (+) = Secondary metabolite detected, (-) = secondary metabolite undetected.		

Table 3. DPPH radical scavenging activity of the methanol extract and ascorbic acid.

Concentration (µg/ml)	Ascorbic acid		Extract	
	Absorbance x 10^{-2}	% Inhibition	Absorbance x 10^{-2}	% Inhibition
100	42.52 ± 0.019^{a}	76 ± 0.012^{a}	35.55 ± 0.020^{b}	69 ± 0.010^{b}
80	55.57 ± 0.020^{a}	69 ± 0.014^{a}	41.67 ± 0.022^{b}	66 ± 0.011^{b}
60	58.57 ± 0.020^{a}	63 ± 0.013^{a}	52.71 ± 0.019^{b}	58 ± 0.014^{b}
40	66.63 ± 0.018^{a}	57 ± 0.013^{a}	58.70 ± 0.022^{b}	55 ± 0.011^{b}
20	70.77 ± 0.021^{a}	53 ± 0.011^{a}	69.90 ± 0.021^{b}	49 ± 0.013^{b}
Note: Different superscript letters behind the numbers indicate a significant difference.				

1.5625 mg/mL. Each well was inoculated with 20 µL of microbial suspension and incubated at 37 °C for 24 h. Subsequently, 20 µL of MTT solution (5 mg/mL) was added and incubated for 30 min. A purple coloration indicated microbial growth, while no color change indicated inhibition. The MIC was defined as having the lowest concentration showing no visible growth. Azithromycin was used as the standard drug (3). All assays were performed in triplicate, and results were expressed as mean ± SEM.

Statistical Analysis
Data were analyzed using Microsoft Excel 2019 and GraphPad Prism version 8.02. Results were expressed as mean ± SEM.

Results and Discussion
Yield Percentage
The calculated % yield of the extraction of the pulverized sample is presented in **Table 1**.

With cold maceration as the method of extraction and methanol as the solvent, 1000 g pulverized sample yielded 100 g of the extract.

Phytochemical Screening of Extract and Pulverized Sample
The stem of *A. leucobotrya,* in both its powdered form and crude extract, was analyzed for the presence of eleven phytochemicals. Five secondary metabolites phenols, tannins, saponins, quinones, and polyphenols were identified in both forms. Numerous medicinal plants are traditionally employed to treat a wide range of ailments (2). Those found to contain these secondary metabolites have been reported to possess antioxidant, hepatoprotective, analgesic, antimicrobial, and anthelmintic activities. The therapeutic effects of plants used in herbal medicine are largely attributed to the presence of these phytochemicals (8).

Antioxidant Activity of Extract
The extract's ability to scavenge hydrogen peroxide and DPPH radicals was used to measure its antioxidant activity.

DPPH Radical Scavenging Activity of the Methanol Extract
The antioxidant activity of *A. leucobotrya* stem barks methanol extract was assessed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in relation to a standard medication (ascorbic acid).

The inhibition values marked with different letters at a given concentration indicate statistically significant differences. DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable free radical that appears as a dark purple crystalline powder in solution. When antioxidants donate a proton to DPPH, it is reduced to DPPH-H, causing a color change from deep purple to pale yellow. The results demonstrate that increasing concentrations of the extract lead to decreased absorbance, while the % of DPPH scavenging activity increases for both the extract and the standard antioxidant, ascorbic acid. The IC_{50} values were 49.96 ± 0.10 µg/mL for the extract and 32.35 ± 0.05 µg/mL for ascorbic acid, with IC_{50} representing the concentration

Table 4. H2O2 scavenging activity of extract and gallic acid.

Concentration (µg/mL)	Gallic Acid		Extract	
	Absorbance x 10^{-2}	%Inhibition	Absorbance x 10^{-2}	%Inhibition
1000	75.76 ± 0.001[a]	69.20 ± 0.002[a]	73.03 ± 0.003[b]	61.32 ± 0.0023[b]
800	72.27 ± 0.012[a]	61.32 ± 0.0012[a]	67.30 ± 0.001[b]	57.86 ± 0.0013[b]
600	68.41 ± 0.002[a]	55.12 ± 0.0018[a]	60.59 ± 0.005[b]	49.06 ± 0.0012[b]
400	61.42 ± 0.006[a]	53.54 ± 0.0013[a]	58.55 ± 0.011[b]	43.45 ± 0.0023[b]
200	57.02 ± 0.003[a]	48.29 ± 0.0015[a]	49.56 ± 0.0021[b]	38.60 ± 0.009[b]

Note: Different superscript letters behind the numbers indicate a significant difference.

Table 5. Summary of the paralysis and death time recorded for the anthelminthic assay of the extract and standard drug.

Concentration (mg/mL)	Extract		Mebendazole	
	Paralysis time (mins)	Death time (mins)	Paralysis time (mins)	Death time (mins)
20	15.08 ± 0.007[a]	36.06 ± 0.003[a]	29.49 ± 0.005[b]	50.13 ± 0.006[b]
10	25.09 ± 0.005[a]	56.08 ± 0.0005[a]	37.48 ± 0.006[b]	80.28 ± 0.005[b]
5	33.65 ± 0.007[a]	117.18 ± 0.009[a]	54.25 ± 0.008[b]	140.09 ± 0.008[b]
2.5	73.04 ± 0.003[a]	216.19 ± 0.009[a]	89.25 ± 0.011[b]	264.71 ± 0.0012[b]
1.25	171.43 ± 0.007[a]	368.08 ± 0.010[a]	170.07 ± 0.002[b]	377.03 ± 0.0013[b]

Note: Different superscript letters behind the numbers indicate a significant difference.

required to inhibit 50% of the free radicals. Although ascorbic acid exhibited a lower IC_{50} and therefore stronger antioxidant activity, the extract's IC_{50} is relatively close, indicating significant antioxidant potential and the possibility of serving as a natural alternative. These results suggest that the extract can effectively neutralize free radicals through the donation of electrons or hydrogen atoms, confirming its antioxidant capacity. Phenolic compounds, commonly found in plants, are known for their ability to chelate metals, reduce oxidative stress, donate hydrogen, and scavenge free radicals, thereby preventing oxidative damage. According to Elmastas *et al*. (2006), as cited in Oboh *et al*, hydroxyl groups in phenolic compounds are particularly important for antioxidant activity (8). Consequently, plants with high polyphenol content are increasingly recognized globally as valuable natural sources of antioxidants (1). Statistical analysis revealed a significant difference ($p < 0.0001$) between ascorbic acid and the extract, further supporting the extract's potential as an effective antioxidant.

Hydrogen Peroxide Scavenging Activity

Inhibition values labeled with different letters at a specific concentration indicate statistically significant differences. The H_2O_2 scavenging assay results revealed that both absorbance and % inhibition decreased as the extract concentration decreased. The IC_{50} values were 151.80 ± 0.094 µg/mL for the standard (gallic acid) and 185.63 ± 0.152 µg/mL for the extract. Although the extract demonstrated notable antioxidant activity, it was lower than that of the standard, further confirming the antioxidant potential of the plant's methanolic stem extract. The antioxidant activity of gallic acid was significantly higher than that of the extract ($p < 0.011$).

Anthelminthic Activity of the Methanol Extract

Adult Earthworm (*Eudrilus eugeniae*) Mortality Activity

Paralysis and death time values labeled with different letters at a given concentration indicate statistically significant differences. The *in vitro* anthelmintic assay provides a rapid method for screening the potential anthelmintic activity of plant extracts. Various concentrations of the methanolic stem extract of *A. leucobotrya* were compared with mebendazole (standard). The extract demonstrated shorter paralysis and death times compared to mebendazole, with shorter times indicating higher anthelmintic activity. *In vitro* activity of the extract may be attributed to the presence of multiple secondary metabolites acting synergistically, as the combined effect of the extract reflects the sum of the actions of its components (10). Worms in the negative control, which contained distilled water, remained active. The anthelmintic activity of the plant extract is likely due to phytochemicals such as tannins, which are known to possess anthelmintic properties (17). In medicinal plants, anthelminthic effects are primarily attributed to secondary metabolites like tannins, alkaloids, flavonoids, and triterpenoids (19), which interfere with energy-generating pathways, causing paralysis and death (18). The extract's activity was significantly higher than that of the standard mebendazole ($p < 0.0021$).

Antimicrobial Analysis

Broth Microdilution

Results from the antimicrobial assay indicated that the extract exhibited activity against the selected microorganisms, including two g-positive bacteria, two g-negative bacteria, and one fungus. The extract inhibited all tested microbes at varying concentrations. The MBC ranged from 12.5 to 25 mg/mL for the bacteria and was 12.5 mg/mL for the fungus. The extract showed differing levels of activity across the tested bacteria, with the fungus being inhibited at lower concentrations than most bacteria, suggesting stronger antifungal activity than antibacterial activity. The choice of microorganisms was informed by the traditional use of the plant; for instance, E. coli is a common cause of diarrhea, and its susceptibility to the extract supports the plant's use in managing diarrheal infections (23). The higher MIC values observed for the *A.*

Table 6. Summary of minimum inhibition concentration and minimum bactericidal concentration results of the extract.

Test organisms	Minimum Inhibition Concentration (MIC)		MBC of Extract (mg/mL)	MIC/MBC Ratio
	Extract (mg/mL)	Azithromycin (mg/mL x 10^{-3})		
S. typhi	25	10	25	1
C. albicans	12.5	10	12.5	1
S. aureus	50	10	25	2
E. coli	25	10	25	1
S. pyogenes	12.5	10	12.5	1

leucobotrya extract compared with the reference antibiotics indicate that the crude extract exhibited lower antibacterial potency. This finding is expected, as crude plant extracts comprise complex mixtures of bioactive and inactive constituents, which may reduce the effective concentration of antimicrobial compounds or alter their activity through synergistic or antagonistic interactions among phytochemicals (24, 25). In contrast, conventional antibiotics are purified single compounds with defined mechanisms of action, enabling greater antimicrobial efficacy at lower concentrations (26). Although purification and isolation of bioactive constituents may enhance antimicrobial activity by increasing the concentration of active compounds, the process may also remove synergistic phytochemical interactions that contribute to the overall biological effect (25, 27). Consequently, further bioassay-guided fractionation and isolation studies are required to identify the compounds responsible for the antibacterial activity of *A. leucobotrya* and to determine whether the observed activity results from individual constituents or synergistic combinations.

Phytochemical screening of *A. leucobotrya* revealed the presence of tannins and saponins, both of which have been widely reported to possess antibacterial properties (19, 20, 24). Tannins exert antimicrobial effects through mechanisms including disruption of bacterial cell wall integrity, inhibition of extracellular microbial enzymes, and chelation of metal ions required for bacterial metabolism (24, 28). Similarly, saponins increase cell membrane permeability by interacting with membrane lipids, leading to leakage of intracellular contents and inhibition of bacterial growth (20, 29). These phytochemicals may therefore contribute to the antibacterial activity observed in the present study; however, their individual roles remain speculative and should be confirmed through bioassay-guided fractionation and structural characterisation of the active constituents (27). To further characterise the antibacterial effect of the extract, the MBC/MIC ratio was evaluated. An MBC/MIC ratio of ≤2 was considered indicative of bactericidal activity, whereas a ratio of ≥4 suggested a bacteriostatic effect (22).

Conclusion

The purpose of this study was to examine *A. Leucobotrya*'s phytochemical components, antioxidant, antibacterial, and anthelmintic properties. The phytochemicals found in the stem bark were extracted using methanol as the solvent. The % yield of the extract was 10.0%. The phytochemicals found to be present in the pulverized sample were phenols, tannins, saponins, quinones, and polyphenols. The methanol extract exhibited antioxidant activity in the DPPH and H_2O_2 assays. It also exhibited anthelmintic activity against *E. eugeniae,* and the anthelmintic activity of the extract was higher than that of the standard drug (Mebendazole). The methanol extract also exhibited antimicrobial activity against both g-negative bacteria (*E. coli, S. typhi*) and g-positive bacteria (*S. aureus, S. pyogenes)* anda fungus (*C. albicans*). The findings of the research have indeed shown that the traditional use of the plant, *A. Leucobotrya, to* cure different ailments is justified, and isolation and purification of individual components of the extract may result in obtaining lead compounds for the synthesis of antioxidant, antimicrobial, and anthelmintic drugs.

Declaration

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Acknowledgment
The authors would like to acknowledge Technicians at Organic Chemistry laboratory of the Department of Chemistry, KNUST. We would also like to acknowledge the KNUST Central laboratory technicians for their immense role in the research. During this study and the preparation of the manuscript, the authors used Claude (Anthropic, USA) to help understand the methodology, edit the English, analyse the factorial design, and check references. AI was not used to generate or alter the experimental data. All AI-assisted output was reviewed and verified by the authors, who take full responsibility for the content of this article

Conflict of Interest
The authors declare no conflict of interest.

Data Availability
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics Statement
Not applicable.

Funding Information
This work received no external funding.

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Additional Information

How to Cite

APA 7th Edition: Opoku Acheampong, L., Ameyaw Baah, K., Oti-Boakye, A., Acheampong, A., Kwabena Dennis, P., Dzidzor Amengor, C. ... Antwi, B. O. (2026). In Vitro Antioxidant, Antimicrobial and Anti-helminthic Activity of Methanolic Extract of Aganope Leucobotrya. Sciences of Phytochemistry, 5(2), 329-335. https://doi.org/10.58920/sciphy0502645

Vancouver: Opoku Acheampong L, Ameyaw Baah K, Oti-Boakye A, Acheampong A, Kwabena Dennis P, Dzidzor Amengor C, Odei J, Tima Sarfo-Mainoo L, Buah P, Antwi BO. In Vitro Antioxidant, Antimicrobial and Anti-helminthic Activity of Methanolic Extract of Aganope Leucobotrya. Sciences of Phytochemistry. 2026;5(2):329-335. https://doi.org/10.58920/sciphy0502645

Harvard: Opoku Acheampong, L., Ameyaw Baah, K., Oti-Boakye, A., Acheampong, A., Kwabena Dennis, P., Dzidzor Amengor, C. ... Antwi, B. O. (2026) 'In Vitro Antioxidant, Antimicrobial and Anti-helminthic Activity of Methanolic Extract of Aganope Leucobotrya', Sciences of Phytochemistry, 5(2), pp. 329-335. doi: 10.58920/sciphy0502645

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