Determination of the Phytochemical, Bioactive Constituents and Antibacterial Activity of Dialium Guineense Stem Extract Against Pseudomonas Aeruginosa and Staphylococcus Aureus Using High Performance Liquid Chromatography (HPLC)

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***Abstract*: This study investigates the bioactive compounds in Dialium guineense stem extract and its effects on Pseudomonas aeruginosa and Staphylococcus aureus. Fresh stems were collected from Enugu South LGA, Enugu State, washed, air-dried, pulverized, and extracted with ethanol using a Soxhlet extractor. Phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, tannins, phenol, reducing sugar, quinone, and steroids. High-Performance Liquid Chromatography identified key bioactive compounds, including Lunamarin (76.3193 mg/ml), Ribalinidine (64.0834 mg/ml), Gallocatechin (48.5752 mg/ml), Resveratrol (11.3824 mg/ml), and Flavones (33.4475 mg/ml), known for antioxidant, anti-inflammatory, and antimicrobial properties. Antimicrobial testing using the Agar Well Diffusion method showed moderate activity, with inhibition zones of 9.4 mm for P. aeruginosa and 5.5 mm for S. aureus at 200 mg/ml. The Minimum Inhibitory Concentration (MIC) was 6.25 mg/ml for S. aureus and 12.5 mg/ml for P. aeruginosa, indicating greater sensitivity of gram-positive bacteria. While the extract exhibits potential as a natural antimicrobial agent, its moderate potency at lower concentrations suggests the need for higher doses, especially against gram-negative bacteria.**

***Keywords*: Resistance, Inhibition, Phytochemical, Extract, Dialium guineense, Antimicrobial, Staphylococcus aureus, Pseudomonas aeruginosa, Bioactive, Antibacterial, Therapeutic, Concentration.**

1. Introduction

Plants play a crucial role in human health, especially in Africa, where over 80% of the population relies on medicinal plants for primary healthcare [1]. In Benin, traditional medicine remains widely practiced due to limited access to modern healthcare, low income, and insufficient medical infrastructure. Herbal medicine is favored for its perceived low toxicity and minimal side effects [2]. Recognizing this, the Ministry of Health in Benin has integrated traditional healers into the national health system [3]. Meanwhile, antibiotic resistance has become a global public health challenge, increasing the urgency to discover new bioactive molecules. Medicinal plants, rich in secondary metabolites like papaverine, berberine and curcumin, offer potential in combating infectious diseases and other health conditions [1, 4].

Dialium guineense, commonly known as black velvet tamarind, is a leguminous plant native to West Africa, belonging to the Fabaceae family. It is widely cultivated for its nutritional and medicinal value [5]. The plant is characterized by its 30-meter height, dense leafy crown, smooth grey bark, and small red gum secretion. Its finely hairy leaves have terminal leaflets and are often elliptical in shape. *Dialium guineense* is known for its abundant phytochemicals, offering nutraceutical benefits [6].

The increasing resistance of pathogenic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* to conventional antibiotics has become a significant public health concern. This resistance limits the effectiveness of standard treatments, leading to persistent infections and increased mortality rates. As a result, there is an urgent need to explore alternative antimicrobial agents, particularly those derived from natural sources. *Dialium guineense*, a plant commonly used in traditional medicine, is known for its rich bioactive compounds [7], yet the potential of the stem extract as an antimicrobial

agent has not been fully explored. This study aims to address this gap by extracting and chemically characterizing the bioactive components of *Dialium guineense* stem using High-Performance Liquid Chromatography (HPLC). Additionally, the antimicrobial efficacy of the extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus* will be evaluated, providing insights into its potential as a natural alternative to combat antibiotic-resistant bacteria.

2. Materials and Method

A. Sample Collection and Treatment

Fresh stems of *Dialium guineense* were collected from Ugwuaji Community farmland in Enugu South L.G.A. Enugu State. The sample was washed immediately with running water and further rinsed with distilled water. The plant sample was air-dried after being sliced into smaller components. Furthermore, the dried *Dialium guineense* stem was grounded into fine powder and stored in a clean sterile container till further analysis.

B. Sample Extraction

The method of Patel et al. [8] was adopted. About 100g of the dried *Dialium guineense* stem samples was extracted using soxhlet apparatus compartment and concentrated with direct heating on a hot plate. Ethanol was used as the solvent for the extraction.

C. Phytochemical Screening of Plant Extract

The method of Velavan [9] was adopted in this study to detect for the presence of alkaloids, saponins, steroids, phenol, flavonoids, tannins and glycosides.

D. Test for Alkaloids

Aliquots of 0.4g of each extract were stirred with 8ml of 2% HCL and the mixture was warmed and filtered. The filtrate 2ml was treated with potassium bismuth (Dragendroff's reagent). Turbidity or precipitation with this reagent was taken as evidence for existence of alkaloids.

E. Test for Saponins

The ability of saponins to produce emulsion with oil was used for the screening test. A total of 20mg of extract was boiled in 20ml of distilled water in a water bath for five minutes and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for froth formation. 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development.

F. Test for Steriods

5 drops of concentrated H_2SO_4 were added to 1ml of each extract in test tubes. A red colouration indicates the presence of steroid.

G. Test for Phenols (Ferric Chloride Test)

10ml of distilled water and 5 drops of 1% ferric chloride solution was added to 1ml of the extract. The colour of the precipitate formed was noted (dark green indicates presence of phenols).

H. Test for Flavonoids

A total of 50mg of each extract were suspended in 100ml of distilled water to get the filtrate. A 5ml of diluted ammonia solution was added to 10ml of filtrate followed by few drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow coloration.

I. Test for Tannins

A total of 50mg of each extracts were boiled in 20ml of distilled H_2O and filtered. A few drops of 0.1%. $FeCl_3$ was added in filtrate and observed for colour change; brownish green or a blue-black coloration was taken as evidence for the presence of tannins.

J. Test for Glycosides

Glycosides are compounds which upon hydrolysis give raise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). 2ml of extract was added to 1ml of glacial acetic acid with one drop of ferric chloride solution. 1ml of concentrated sulfuric acid was added and observed for a reddish-brown coloration at the junction of two layers and the bluish green colour in the upper layer which indicates the presence of glycosides.

K. Bioactive Compounds Using High Performance Liquid Chromatography Analysis

High performance liquid chromatography (HPLC) analysis was performed using Shimadzu LC-10AD dual binary pumps, Shimadzu CTO-10AS column oven and Shimadzu Prominence SPD-20A UV/V is detector. The analysis was performed using a C-12 normal phase column (Phenomenex, Gemini 5 µ, 200 mm length × 4.8 mm internal diameter). The mobile phase consisted of acetic acid-acidified deionized water (pH 2.8) as solvent A and acetonitrile as solvent B at a flow rate of 0.8 mL/min. The column was equilibrated with 5% solvent B for 20 min after each injection of samples. The column temperature was set to 38°C and the injection volume was 20 µL. The wavelengths were set to 280 nm for the detection of phenolics, Phenolic compound identification and quantification were performed by comparing respective retention times and peak areas with pure standard compounds utilizing the method of external standards to construct calibration curve. Gradient elution was executed as follows: 0-5 min, 5-9% solvent B; 5-15 min, 9% solvent B; 15-22 min, 9-11% solvent B; 22-38 min, 11-18% solvent B; 38-43 min, 18-23% solvent B; 43-44 min 23-90% solvent B; 44-45 min, 90-80%, solvent B; 45-55 min

L. Isolation and Identification of the Test Organisms

The following organisms were isolated from differed Clinical samples submitted to Bacteriology Department of Christian Medical Laboratory, Agbani Road Enugu:

Staphylococcus aureus, *Pseudomonas aeruginosa* (both from wound isolates). They were subculture and identified by their cultural characteristics and biochemical properties. The isolates were finally re-sub-cultured into different culture bottles for further use.

M. Preliminary Screening of the Bacterial Organisms

Gram staining and biochemical tests were carried using the method established by Naveena and Joy, [10].

N. Antimicrobial Activity

A total of 0.4g of the *Dialium guineense* stem extract was dissolved with 2ml of 50% dimethyl sulfuroxide to give a stock concentration of 200mg/ml. A double fold dilution was further done to obtain other concentrations at 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml respectively.

O. Agar Well Diffuse Method

0.1m of the organism already matched with MacFarland`s turbidity standard were inoculated onto Mueller Hinton agar plates. A sterile cork borer (6mm diameter each) was used to make seven wells for each concentration of the extracts containing cultures of the test isolates. 0.1ml of each concentration of 3.125, 6.25, 12.5, 25, 50, 100 and 200mg/ml of the extracts were then introduced into seven wells using sterile Pasteur pipettes. Ciprofloxacin was used as a positive control. The culture plates were allowed to stand on the working bench for 30mins for pre-diffusion and were then incubated at 28^0C for 24h. After 24h, antibacterial activity was determined by measuring the diameter zones of inhibition in mm [10].

P. Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extract

The concentrations used in the antimicrobial susceptibility test were diluted further to get different concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.25mg/ml which were incorporated into various test tubes, 0.1ml of the test organism was introduced and spread on each plate containing Mueller Hinton agar 6mm wells were cut using sterile cork borer and each filled with 0.1m of extracts. The plates were incubated at 28^0C for 24hr and the resultant zone of inhibitions was measured. The least concentration of the extracts that inhibits the growth of the test organisms were designated as the Minimum Inhibitory Concentrated (MIC) [10].

3. Result

A. Phytochemical Evaluation of Dialium Guineense Stem Extract

The result of the phytochemical constituents of *D. guineense* stem extract are tabulated in Table 1.

Table 1
Phytochemical composition of dialium guineense stem extract

Phytochemical Components	Indication
Alkaloids	+++
Saponin	+++
Glycosides	+
Flavonoids	+
Tannins	+++
Phenol	+
Steroids	+

Key: + (low); ++ (moderate); +++ (High); - (Absence)

B. Bioactive Composition of Dialium Guineense Stem Extract

Various bioactive compounds were identified in *Dialium guineense* stem extract which contributed to its medicinal and therapeutic significance.

Table 2
Bioactive composition of dialium guineense stem extract

Bioactive Compounds	Concentration (mg/ml)
Resveratol	11.3824
Catechin	22.5308
Ribalinidine	64.0834
Flavoones	33.4475
Flavan-3-Ol	30.4883
Flavone	12.656
Aglycone	41.6918
Lunamarin	76.3193
Gallocatechin	48.5752
Isoflavonoids	7.2342
Kaemferol	27.9606
Kaempferol	0.7358

C. Biochemical Reaction of the Bacterial Organisms

The biochemical test reaction identifies the presence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with their various characteristics (Table 3).

Table 3
Identification and biochemical characterization of the isolates

Test	Pseudomonas-aeruginosa	Staphylococcus sp.
Gram Staining	-v short rod in pairs	+v cocci in clusters
Biochemical Test		
Catalase Test	+	+
Oxidase Test	+	-
Coagulase Test	-	-
Methyl Red Test	+	+
Citrate Test	-	-
Voges-proskauer test	-	-
Indole Test	+	-
Sugar Fermentation Test		
Glucose	-	A
Mannitol	A	A
Lactose	-	A

Key: A (Acid production); +v (positive); -v (negative)

D. Inhibition Zone Diameter (mm) of Dialium Guineense Stem Extract on the Test organisms

The extract showed inhibition to the microorganisms, whereby the standard drug showed more efficacies to the isolates than the plant extract. The extract showed highest inhibition rate at higher concentration (Table 4).

Table 4
Inhibition zone diameter (mm) of dialium guineense stem extract

Conc. (mg/ml)	Pseudomonas aeruginosa	Staphylococcus aureus
200	9.4mm	5.5mm
100	6.2mm	5.2mm
50	3.5mm	4.0mm

25	0.8mm	2.1mm
12.5	0.8mm	1.6mm
6.25	-	0.4mm
3.125	-	-
Cipro.	14.0mm	16mm

Key: Conc. (Sample Concentration); Cipro. (Ciproflaxacin-Standard drug)

E. Minimum Inhibitory Concentration (MIC) of Dialium Guineense Stem Extract (mg/ml)

The table showed the minimum inhibitory concentration of the *Dialium guineense stem* extract and from the result, the identified microorganisms were inhibited at different extract concentrations (Table 5).

Table 5
Minimum inhibitory concentration (MIC) of dialium guineense stem extract (mg/ml)

Test Organism	MIC (mg/ml)
Staphylococcus aureus.	6.25
Pseudomonas aeruginosa	12.5

4. Discussion

The bioactive component of natural plant has been extracted for various scientific purposes, including the synthesis of herbal medicine and modern drugs. In this developing world, there is a little bit diversion of interest over the use of modern drugs (over the counter drugs) to the application of natural plant's extract for the treatment of human diseases and infections. This singular application is due to the presence of these bioactive compounds which are medicinally important [7, 14]. In this present study, the phytochemical analysis reveals the presence of alkaloids, saponins, flavonoids, tannins, phenol, Glycosides, reducing sugar, quinone and steroids (Table 4.1).

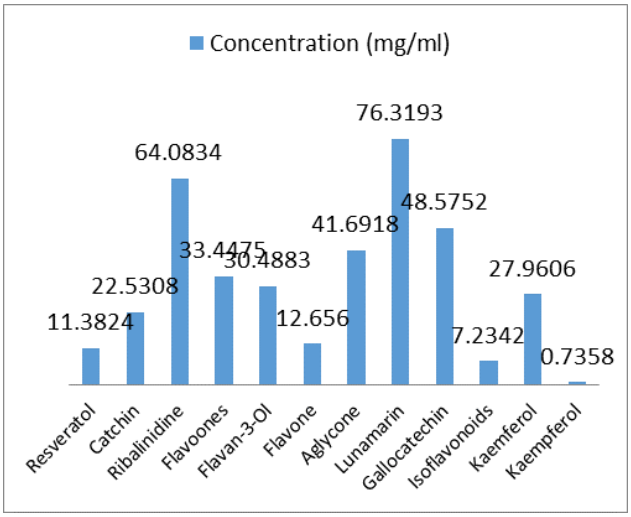

Fig 1: The figure indicates the variation of the concentration of the identified bioactive compounds (chemicals) in dialium guineense Stem Extract

This result was supported with key bioactive compounds present in the stem extract, including Lunamarin (76.3193 mg/ml) and Ribalinidine (64.0834 mg/ml), which show high concentrations, suggesting significant pharmacological activities. Flavoones (33.4475 mg/ml), flavan-3-Ol (30.4883 mg/ml), gallocatechin (48.5752 mg/ml) and aglycone (41.6918 mg/ml). Interestingly, resveratrol (11.3824 mg/ml), known for its cardio-protective properties, also adds to the therapeutic potential. The extract's broad spectrum of bioactive compounds, especially its high flavonoid content, supports its traditional use in herbal medicine for treating various ailments. A previous study by Oki *et al*. [11] showed similar result as obtained in this present study. Abu *et al*. [12] found that *D. guineense* contains high levels of phenols, flavonoids, saponins and tannins, which may explain why it is used in traditional medicine.

The biochemical test identifies the presence of two pathogenic bacteria organisms which include *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 4.2). The inhibition zone diameter for *Dialium guineense* stem extract against Pseudomonas *aeruginosa* and *Staphylococcus aureus* highlight its antimicrobial potential, though its effectiveness varies with concentration. At the highest concentration (200 mg/ml), the extract shows moderate activity, inhibiting *P. aeruginosa* (9.4 mm) more than *S. aureus* (5.5 mm). However, as concentration decreases, the inhibition weakens, with significant reduction observed at 50 mg/ml and below. At 3.125 mg/ml, no inhibition was observed for either organism, indicating a concentration-dependent response. This present result is similar to the result of Ololade *et al*. [13]. Nnadi *et al*. [14] found that methanol *D. guineense* fruit coat extract had stronger antibacterial property than ethanol *D. guineense* stem bark extract.

Ciprofloxacin, the standard drug, exhibited much stronger activity with inhibition zones of 14.0 mm and 16 mm against *P. aeruginosa* and *S. aureus*, respectively, demonstrating that while the stem extract has antibacterial properties, it is less potent than conventional antibiotics. The minimum inhibitory concentration (MIC) of *Dialium guineense* stem extract in table 4.4 reveals its antimicrobial efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The MIC for *S. aureus* is 6.25 mg/ml, indicating that it is more sensitive to the extract compared to *P. aeruginosa*, which has a higher MIC of 12.5 mg/ml. This suggests that the extract is more effective against gram-positive *S. aureus* at lower concentrations.

The extract's more pronounced effect on *P. aeruginosa* suggests that it could be more effective against gram-negative bacteria. The overall results point to the extract's potential as a natural antimicrobial agent, but its efficacy is limited at lower concentrations. While the extract shows antimicrobial activity, its relatively high MIC values suggest moderate potency [13]. It could serve as a natural antimicrobial agent, especially for *S. aureus* infections, but might require higher doses for gram-negative bacteria like *P. aeruginosa*. With this evidence identified in this study, further clinical studies are recommended to show the medicinal efficacy of the plant extract on *In-vitro* and *In-vivo* analysis.

5. Conclusion

In conclusion, this study highlights the potential of *Dialium guineense* stem extract as a natural antimicrobial agent, particularly against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Rich in bioactive compounds like Lunamarin and Ribalinidine, the extract exhibited moderate antibacterial activity, with stronger effects on *P. aeruginosa* at higher concentrations. The MIC results showed greater efficacy against *S. aureus*, though less potent than ciprofloxacin. While the findings suggest therapeutic potential, the extract's reduced effectiveness at lower concentrations indicates the need for further in-vitro and in-vivo research to optimize its dosage and fully explore its medicinal applications.

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