

Crescentia kujete Leaves Extract: A Source of Bioactive Compounds for the Development of Antibacterial Agents

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Abstract- This study focused on the phytochemical screening and antibacterial analysis of *Crescentia kujete* leaves extract, a plant widely used in traditional medicine. The leaves were extracted using ethanol through maceration, and the crude extracts were subjected to phytochemical screening and antibacterial testing against some selected bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Shigella*). The qualitative phytochemical screening revealed the presence of saponins, flavonoids, tannins, alkaloids, steroids, and cardiac glycosides with carbohydrates and proteins, while anthraquinones were absent. The antibacterial activity results showed that the extract exhibited inhibitory effects against the tested bacteria, with zones of inhibition ranging between 0.40 mm and 1.95 mm. The Minimum Inhibitory Concentration (MIC) demonstrated sensitivity of *S. aureus* and *B. subtilis* at higher concentrations, while *E. coli* and *Shigella* were comparatively more resistant. Similarly, the Minimum Bactericidal Concentration (MBC) results revealed that bactericidal activity varied with bacterial type and concentration, indicating both bacteriostatic and bactericidal potentials of the extract. The findings validate the ethnomedicinal use of *Crescentia kujete* leaves and highlight their potential as a source of natural antibacterial agents.

Index Terms- *Crescentia kujete*, Leaves, Extract, Phytochemical, Antibacteria

I. INTRODUCTION

The growing prevalence of antimicrobial resistance has created an urgent need for alternative sources of antibacterial agents. Conventional antibiotics are increasingly failing against multidrug-resistant pathogen, which poses a serious threat to public health worldwide. As a result, scientists are turning their focus toward natural sources, particularly medicinal plants, as promising alternatives due to their rich bioactive profiles and historical use in traditional medicine [1-4]. Medicinal plants are known to contain diverse phytochemicals such as alkaloids, flavonoids, tannins, phenols, and terpenoids, many of which possess antimicrobial activity. These compounds often act through different mechanisms such as disruption of bacterial membranes, inhibition of enzymatic pathways, or

interference with nucleic acid synthesis. Phytochemical screening therefore provides a vital foundation for identifying plants with potential therapeutic applications [5-8]. The calabash tree (*Crescentia kujete* L.), a member of the family *Bignoniaceae*, is one such medicinal plant that has attracted increasing attention. Traditionally, its leaves, bark, and fruit have been used in the treatment of respiratory infections, wounds, skin diseases, and inflammatory conditions in several cultures. This ethnobotanical background underscores the relevance of subjecting the plant to systematic phytochemical and antibacterial evaluation [9]. Recent investigations on *Crescentia kujete* have revealed the presence of flavonoids, saponins, tannins, and phenolic compounds, which are linked to antimicrobial and antioxidant activities. For example, both in vitro and in silico studies have provided evidence that the leaves may contain metabolites with significant pharmacological potential [10]. Despite this promising evidence, studies on the antibacterial properties of *C. kujete* remain limited and often differ in methodology, solvents, and bacterial strains tested. Such variations highlight the need for controlled experimental studies, especially in undergraduate research contexts, to provide reproducible data that can validate traditional claims and support potential pharmaceutical applications. Solvent-based extraction has proven to be effective in isolating active plant metabolites. Comparative studies show that polar solvents such as methanol and ethanol often yield higher concentrations of bioactive compounds from leaves, which correlates with stronger antibacterial effects. This reinforces the rationale for focusing on *C. kujete* leaves to assess both their phytochemical profile and antibacterial activity [11].



Plate 1: *Crescentia cujete* Leaves

II. MATERIALS AND METHOD

All chemicals and solvents were obtained from LobaChemie and used as received. Mular Hilton nutrient agar and broth were used for the antibacterial analysis. The test organisms were obtained from the Department of Biological Science, Sokoto State University. The microorganisms are standard laboratory strains of *Staphylococcus aureus* (gram +ve), *Escherichia coli* (gram -ve), *Bacillus subtilis* (gram +ve), and *Shigella* (gram -ve).

Sample collection and treatment

The Calabash leaves were collected in the month of July, 2025 from Tunga Area, of Wamakko Local Government Sokoto, Sokoto State, Nigeria. The leaves were rinsed using clean water and dried under shade for 7 days. The dried leaves were transferred into a clean mortar and pestle and pound to a fine powdered, and it was sieved to have complete powder. The powdered sample was transferred into a water free and air tight polythene bag and stored for further analysis.

Determination of Moisture Content

The leaves were taken in a pre-weighed empty dish (W_0) and their resulting weight was denoted by (W_1). The dish containing the leaves sample was transferred into an oven at $100\text{ }^\circ\text{C}$ for 24 hours then removed, and the weight of the dish containing the dried sample was determined and denoted by (W_2). The percentage moisture content of the leaves was calculated using equation below.

$$\% \text{ moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \dots \dots \dots (1)$$

Where W_0 = weight of the empty dish

W_1 = weight of empty dish + sample

W_2 = weight of to the empty dish + dry sample.

Extraction of *Crescentia cujete* Leaves

The powdered sample (90 g) was measured and put into a clean 1000 cm^3 conical flask. Ethanol (200 cm^3) was added and the flask was properly sealed with aluminum foil to minimize solvent evaporation. The maceration process was allowed to proceed for 24 hours with occasional shaking for thorough mixing of the solvent and plant matrix. After sufficient soaking and extraction, the mixture was carefully filtered using local sieve into a sterile beaker to separate the filtrate from the plant residues. This ensured the recovery of a clear extract containing the soluble constituents of the *Crescentia cujete* leaves. The filtrate obtained was concentrated by gentle evaporation of excess ethanol at controlled temperature conditions until a crude extract was obtained. The extract was stored in a labeled airtight container prior to further phytochemical screening and bioactivity analysis. The percentage yield of the extracts was calculated using equation below:

$$\% \text{ extract yield} = \frac{\text{Weight of extract}}{\text{Initial weight of sample}} \times 100 \dots \dots \dots (2)$$

Qualitative Phytochemical Screening of Extract.

The tests for flavonoids, tannins, saponins, steroids/triterpenoids, alkaloids, cardiac glycosides and anthraquinones were carried according to the methods described by [12-18].

Antibacterial Susceptibility Tests

The antibacterial test was conducted using the petri dish plate method described by [19]. The Nutrient Agar plates prepared according to manufacturer's instruction were allowed to solidify for 15 minutes at room temperature and incubated without inoculum for 24 hours at $37\text{ }^\circ\text{C}$ to ensure the sterility of the medium. The Nutrient Agar plates were flooded with 1 ml of the inoculum and the excess was removed using Pasteur pipette. 4-6 wells (cups) of about 6 mm in diameter were cut on each Nutrient Agar plate using a sterile cork borer and the agar plugs were removed using a sterile ampoule file. The extract solution (0.1 mL) was placed in each of the wells and were allowed to settle for two hours at room temperature and then incubated for 24 hours at $37\text{ }^\circ\text{C}$. The inhibition zone was observed and then recorded in millimeters using a transparent ruler. Standard antibiotics were used.

Minimum Inhibitory Concentration (MIC)

This was carried out as described by [20]. Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration where no visible turbidity would be observed in the test tubes. The MIC was determined for the micro-organisms were prepared using the broth dilution technique the stock solution (20 mg/ml) of the fruit extract was prepared by dissolving 0.2g of the fruit extract in 10 ml DMSO three concentration were prepared (0.25mg/ml, 0.50mg/ml, 0.75mg/ml) from the stock solution using serial dilution technique and later inoculated with 0.2ml, suspension of the test organism, After 24 hours incubation at 37 °C, the tubes were observed for turbidity. The lowest concentration where no turbidity was observed was determine and noted as the Minimum Inhibitory Concentration (MIC).

Minimum Bactericidal Concentration (MBC)

The minimal bactericidal concentration was determined from broth dilution test resulting from the MIC tubes as described previously (2.4.1) by inoculating the content of each test tube on a nutrient agar plate. The plates were then incubated at 37 °C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration [20].

III. RESULTS AND DISCUSSION

Percentage Moisture Content of *Crescentia kujete* Leaves

The details of the percentage moisture content obtained from *Crescentia kujete* leaves were given in Table 1. The leaves had a percentage moisture content of 79.00 %.

Table 1: Percentage moisture content of *Crescentia kujete* leaves (%)

Sample	W ₀	W ₁	W ₂	Moisture content (%)
<i>Crescentia kujete</i> leaves	5.00	68.00	18.25	79.00 %

Key: - W₀ = Weight of empty dish, W₁ = Weight of empty dish + sample, W₂ = Weight of empty dish + dry sample

Extraction of *Crescentia kujete* Leaves

The mass of the extract and percentage yield obtained from *Crescentia kujete* leaves were given in Table 2. The extract has a mass of 24.50 g with percentage yield of 27.22 % as shown in the table below.

Table 2: Mass of Extract (g) and percentage yield of *Crescentia kujete* leaves (%)

Extract	Original mass (g)	Mass (g) of extract	Percentage yield (%)
Ethanol	90.00	24.50	27.22

Qualitative Phytochemical Screening of *Crescentia kujete* Leaves Extract

The results of the qualitative phytochemical screening of the extract obtained were shown in Table 3. Saponins, flavonoid, tannins, alkaloid, steroids, carbohydrates and protein, were detected, while anthraquinones were not detected.

Table 3: Qualitative Phytochemical Screening of *Crescentia kujete* leaves extracts

Phytochemicals	Ethanol
Carbohydrates test	
(a) Molich's test	+
(b) Fehling test	+
Protein test	
(a) Xanthoprotic test	+
Saponins	
(a) Froth's test	+
Flavonoids	
(a) Alkaline test	+
(b) Ferric chloride test	+
(c) Shinoda's Test	+
Tanins	
(a) Ferric chloride test	+
(b) Lead acetate test	+
Alkaloid	
(a) Mayer's test	+
(b) Wagner's reagent	+
(c) Hager's test	+
Steroids and Inter-terpenoids test	
(a) Salkowski's test	+
(b) Libermann-	+
Richard's test	
Anthraquinone	
(a) Borntrager's test	-

Cardis glycoside's test +

Keys: - (+) = detected (-) = not detected

Antibacterial Susceptibility Test

The results obtained from the antibacterial test were shown in table 4a – c.

Table 4a: Antibacterial activity of *Crescentia kujete* leaves extracts

Concentration (mg/mL)	Zone of Inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>Shigella</i>
25	0.00	0.00	0.00	0.62
50	0.40	0.00	1.03	0.85
75	1.30	0.00	1.75	1.03
100	1.40	0.00	1.95	1.05

Key: - *S. aureus* = Staphylococcus aureus, *E. coli* = Escherichia coli, *B. subtilis* = Bacillus subtilis

Table 4b: Antibacterial activity of standard antibiotic (Ceftriaxone)

Antibiotic Conc. (mg/mL)	Zone of Inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>Shigella</i>
Ceftriaxone (50 mg/mL)	4.26	4.85	4.05	4.67

Key: - *S. aureus* = Staphylococcus aureus, *E. coli* = Escherichia coli, *B. subtilis* = Bacillus subtilis

Table 4c: MIC and MBC of *Crescentia kujete* leaves extract

Test Organisms	MIC (Mg/mL)	MBC (Mg/mL)
<i>S. aureus</i>	75.00	75.00
<i>E. coli</i>	75.00	75.00

<i>B. subtilis</i>	50.00	50.00
<i>Shigella</i>	50.00	50.00

Key: - MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration

IV. DISCUSSION

The results obtained from the moisture content and solvent extraction of *Crescentia kujete* leaves revealed percentage moisture content of 79.00 %, indicating that the leaves have high water content (Table 1). The mass of the extract was recorded as 24.50 g, with a percentage yield of 27.22 % (Table 2). The phytochemical screening of *Crescentia kujete* leaves revealed the presence of important bioactive compounds such as saponins, flavonoids, tannins, alkaloids, steroids, and cardiac glycosides with carbohydrates and proteins, while anthraquinones were absent (Table 3). The presence of these secondary metabolites is significant because they are associated with a wide range of pharmacological properties. Flavonoids and tannins are well-known for their antioxidant and antimicrobial roles, contributing to bacterial cell wall disruption and enzyme inhibition [21]. Alkaloids, which were also detected, have been reported to possess broad-spectrum antibacterial activity through mechanisms that involve DNA intercalation and interference with protein synthesis [22]. The absence of anthraquinones in this study is consistent with reports that their occurrence is species and solvent-dependent, often requiring non-polar solvents for effective extraction. Similar findings were reported by [23], who observed flavonoids, alkaloids, and saponins as the dominant compounds in *C. kujete* leaves using ethanol extracts [23]. These results suggest that the bioactivity of the extract is likely due to synergistic interactions between flavonoids, tannins, and alkaloids.

The antibacterial activity of *Crescentia kujete* leaves extract was investigated against four test organisms: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Shigella* species. The results, as presented in Tables 4a and 4b, revealed that the plant extract has activity against the selected bacteria with zone of inhibition ranging between 0.40 mm to 1.95 mm while the zones of inhibition produced by the standard antibiotic (Ceftriaxone, 50 mg/cm³) ranged between 4.05 mm and 4.85 mm across all test organisms. These values, although relatively small, demonstrate that the antibiotic maintained inhibitory activity under the test conditions. Comparatively, the inhibition zones observed for *C. kujete* extracts (data from MIC and MBC) indicate that

the plant exhibits promising antibacterial potential, though at higher concentrations than the standard drug.

The MIC and MBC results showed that *B. subtilis* and *Shigella* were more sensitive to the plant extract, both having MIC and MBC values of 50 mg/cm³. In contrast, *S. aureus* and *E. coli* required higher concentrations (75 mg/cm³) to achieve inhibitory and bactericidal effects. This differential susceptibility suggests that the phytochemicals present in *C. kujete* leaves may have stronger activity against Gram-positive *B. subtilis* and Gram-negative *Shigella*, compared to *S. aureus* and *E. coli*. Such variation may be attributed to differences in cell wall structure, permeability barriers, and resistance mechanisms between bacterial strains [24]. The observation that the MIC and MBC values were equal for all organisms suggests that the extract possesses bactericidal rather than bacteriostatic activity. A compound is considered bactericidal when MBC values are close or equal to MIC values [25]. This finding is consistent with earlier reports that highlighted the broad-spectrum bactericidal activity of *C. kujete* and other ethnomedicinal plants rich in secondary metabolites such as flavonoids, tannins, and alkaloids [26].

The activity of *C. kujete* may be linked to its phytochemical constituents, which are known to interfere with bacterial cell wall synthesis, protein function, and nucleic acid replication [27]. Previous studies have reported that flavonoids and tannins disrupt microbial cell membranes, while alkaloids may inhibit nucleic acid synthesis [28]. These mechanisms collectively explain the broad inhibitory action observed in this study. Although the plant extract required higher concentrations compared to the standard antibiotic, its antibacterial activity is still worthy. This aligns with traditional claims of *C. kujete* use in managing infections such as respiratory ailments, diarrhea, and skin diseases [29]. Furthermore, the ability of the extract to exhibit activity against both Gram-positive and Gram-negative organisms supports its potential as a source of bioactive compounds for the development of novel antibacterial agents [30, 31]. In summary, the results demonstrate that *Crescentia kujete* leaves extract possesses antibacterial activity, with significant bactericidal effects against both Gram-positive and Gram-negative bacteria. The findings provide scientific justification for the ethnomedicinal use of the plant and highlight its potential for future drug discovery.

V. CONCLUSION

This study has shown that *Crescentia kujete* leaves contain a wide range of phytochemicals, including flavonoids, tannins, saponins, alkaloids, steroids, and cardiac glycosides, which are associated with antibacterial activities. The absence of anthraquinones suggests that not all classes of secondary metabolites are present in the leaves, which may influence the spectrum of bioactivity observed. The antibacterial evaluation revealed that the extracts exhibited both bacteriostatic and bactericidal effects, with stronger activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, while Gram-negative bacteria like *Escherichia coli* and *Shigella* were comparatively resistant. These findings validate the ethnomedicinal claims regarding the use of *Crescentia kujete* leaves in treating infectious diseases, though the activity was moderate compared to standard antibiotics. The results highlight the potential of *Crescentia kujete* leaves as a natural source of antibacterial agents. However, the crude nature of the extract, coupled with variation in bacterial susceptibility, suggests that further studies are required to isolate and characterize the specific bioactive compounds responsible for the observed effects.

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