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Research Article

Preliminary Screening Of The Antisickling Properties Of Brillantaisia Owariensis Leaf Extracts

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ABSTRACT

The integration of phytomedicines into modern pharmacotherapy represents a critical frontier in drug discovery, offering diverse bioactive compounds with complex mechanisms of action for disease management. Brillantaisia owariensis is widely utilized in traditional medicines to manage of sickle cell disease, however, scientific validation of this ethnobotanical claim remains scarce. This study evaluated the antisickling potential of Brillantaisia owariensis leaf extracts through phytochemical analyses, alongside antimicrobial assays. The investigation was based on the premise that specific bioactive constituents can inhibit erythrocyte sickling and that certain microbial pathogens contribute to vaso-occlusive crises in sickle cell patients. Qualitative phytochemical screening confirmed the presence of phenolics, cardiac glycosides, saponins, steroids, and gums. Quantitative analysis showed that the plant extract contained phenolics (95.8 ± 0.0015mg/g), flavonoids (122.2 ± 0.0026mg/g) and cardiac glycosides (163.8 ± 0.0031mg/g) for the ethanolic extract and (59.6 ± 0.001mg/g), (43.6 ± 0.002mg/g), and (118 ± 0.0025mg/g) respctively for the n-hexane extract. Both extracts exhibited robust antimicrobial activity, producing zones of inhibition equivalent to 60 –70% of ciprofloxacin control, and exhibited activity against typed bacterial strains (S. aureus NCTC8532, B. subtilis NCTC10400, P. aeruginosa NCTC10662, E. coli NCTC10418) as well as clinical microbial isolates of S. aureus, E. coli, and P. aeruginosa.

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Statistical analysis showed a significant difference ($p < 0.05$) between the extracts, establishing the superior activity of the ethanolic extract over the n-hexane extract. These findings provide compelling scientific evidence supporting the traditional use of Brillantaisia owariensis in the management of sickle cell disease and warrants further investigation.

INTRODUCTION

Phytomedicine, known as the science and practice of using plant-derived therapeutics for the prevention and management of diseases, has re-emerged as a vital component of modern healthcare, rooted in centuries of ethnobotanical knowledge and now validated by rigorous pharmacological research. Phytomedicine bridges traditional wisdom and contemporary biomedical science[1]. In today's therapeutic landscape, phytomedicine functions as a complementary approach to conventional medicine, It addresses critical gaps in healthcare delivery, particularly in chronic disease management, palliative care, and resource-limited settings where access to synthetic pharmaceuticals may be constrained[2]. Plant-based formulations often exhibit multi-target mechanisms, acting on several physiological pathways simultaneously, which makes them valuable in complex conditions such as diabetes, hypertension, inflammatory disorders, and antimicrobial resistance etc[3,4]. Recognized by the World Health Organization as a pillar of primary healthcare for over 80% of populations in developing countries, phytomedicine is no longer viewed as "alternative" but as integrative[5]. Consequently, it is regarded as an integral component of integrative medicine[6].

Brillantaisia owariensis

Fig.1 leaves of *Brillantaisia owariensis* P. Beauv. Family (Acanthaceae).

Other names: giant blue African salvia, Tropical giant salvia

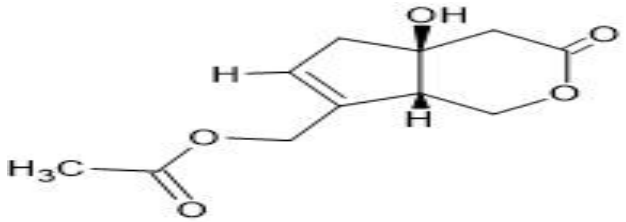
Local names;

Abureni: Ozuzu-oghogho, (Abua/Odual)

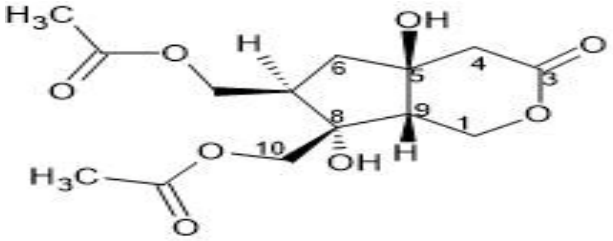
Ogbia: Obom-ato , ogbua-emu (Bayelsa)

Brillantaisia owariensis P. Beauv. is a perennial shrub native to tropical Africa, with a distribution spanning Central and West Africa, It typically grows in moist areas, along river banks and in coastal areas reaching heights of up to 2-8 meters. It holds a prominent place in indigenous pharmacopeias, particularly in regions such as the Democratic Republic of Congo, Nigeria, and Angola, where it is traditionally valued as a blood-tonic for managing anaemia and related complications[7-9]. Phytochemical analyses of *B. owariensis* have unvailed diverse array of

bioactive compounds. Among these are iridolactones including the novel compounds *owariensisone* B, C and D isolated by Foning[10] as well as flavonoids, phenolics, saponins and cadiac glycoside[11].



Owariensisone B



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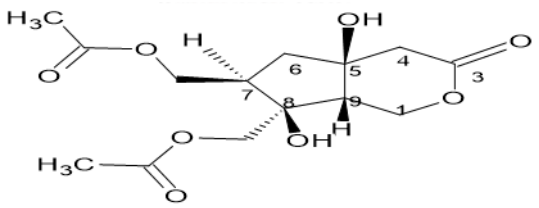

Owariensisone D

In this context, the present study aims to evaluate the preliminary antisickling properties of *Brillantaisia owariensis* through phytochemical screening and assessment of its antimicrobial activity. Previous studies have demonstrated that specific phytochemical constituents contribute significantly to antisickling effects. For instance, cardiac glycosides and alkaloids have been reported to regulate ionic balance, particularly through modulation of Na^+/K^+ pumps, thereby preventing cellular dehydration that promotes hemoglobin S (HbS) polymerization[12]. Phenolic compounds exhibit potent antioxidant properties, mitigating oxidative stress a key factor in erythrocyte dehydration and subsequent sickling[13]. Coumarins and anthocyanins possess anti-inflammatory and vasoprotective properties that may reduce the adhesion of sickled erythrocytes to vascular endothelium, thereby decreasing the frequency of vaso-occlusive crises[14]. Saponins contribute to membrane stabilization by enhancing the structural integrity of red blood cell membranes, reducing osmotic fragility, and preventing premature hemolysis[15]. Additionally, anthocyanins and flavonoids have been reported to inhibit the polymerization of deoxygenated HbS by increasing oxygen affinity and stabilizing the oxyhemoglobin state[16]. Complementarily, some phytochemicals have been reported to demonstrate antimicrobial properties these includes; phenolics, known to complex with bacterial cell walls and disrupt microbial membranes; alkaloids, are

reported to correlate with the inactivation of microbial adhesions, enzymes, and cell proteins; and terpenoids, which facilitate the penetration of the lipophilic cell wall of microorganisms[17,3]. Therefore, the presence of these phytochemicals in *B. owariensis* would serve as a biochemical indicator of its antisickling potential and provide justification for the claim made by locals on its use to manage sickle cell disease complications and anemia.

MATERIAL AND METHOD

Materials

Typed organisms *Staphylococcus aureus* (NCTC 8532), *Bacillus subtilis* (NCTC 10400), *Pseudomonas aeruginosa* (NCTC 10662), and *Escherichia coli* (NCTC 10418)). clinicals isolates of *S. aureus*, *E. coli*, and *P. aeruginosa*. Ethanol (BDH Chmicals LTD), n-Hexane (LiChrosolv, Germany), Distilled water, Mercuric (II) Chloride, Potassium iodide (BDH Chemicals LTD England), Hydrochloric acid (LOBA Chemie Mumbai, India), Ferric chloride (BDH Chemical Ltd. England), Ammonia solution (LOBA Chemie Mumbai, India), Benzene (LOBA Chemie Mumbai, India), Sodium hydroxide (LOBA Chemie Mumbai, India), Chloroform(Molychem, India), Sulphuric acid(LOBA Chemie Mumbai, India), Gacial acetic acid (LOBA Chemie Mumbai, India). (all reagents used in this study were not further purified)

Methodology

Plant Collection and Identification

Fresh leaves of *Brillantaisia owariensis* were harvested from Otuasega community, Ogbia Local Government Area, Bayelsa State, Nigeria (Latitude 4.92520°N, Longitude 6.40310°E) in August, 2025 and identified by a botanist of the Department of Biological Sciences, Niger Delta University, Dr. Ayo Oyedeji as *Brillantaisia owariensis* P. beauv. A voucher specimen of the plant was deposited in the university's herbarium with number (NDUP/0166) for further reference.

Extraction of the plant material

Air-dried and powdered plant material (300 g X 2) was extracted with Ethanol and n-hexane (3L) respectively by maceration process. The extracts were concentrated using a water bath at low temperature (40-50 °C) and dried to a constant weight in a desiccator and the percentage yield determined.

Qualitative phytochemical screening

The freshly prepared plant extracts were qualitatively tested for the presence of phenolics (Ferric chloride test), tannins (Ferric chloride test), phlobatannins (Hydrochloric acid test), flavonoids (Shinoda test), anthocyanins (pH-dependent colour reaction test), terpenoids (Salkowski test), saponons (Frothing test), cardiac glycosides (Keller-Kiliani test), coumarins (sodium hydroxide test), alkaloids (wagners reagent), gums and fixed oils (Precipitation test) using standard colorimetric and precipitation protocols described by Harborne, Trease & Evans and Ismail *et al.,*[18-20] .

Quantitative Phytochemical Determination

Total Phenol Content

Principle: Phenolic compounds react with phosphomolybdic acid in the Folin–Ciocalteu to form a blue-colored molybdenum complex under alkaline condition.

The total phenolic content (TPC) of the extracts was quantified using the Folin–Ciocalteu colorimetric method, based on modified procedures reported by Singleton et al., and Demiray et al.,[21-22]. A standard calibration curve

was established using gallic acid solutions prepared at concentrations between 100 and 1000 µg/mL. For the standards, Folin–Ciocalteu reagent 1.5 mL was added to each solution and allowed to react at room temperature for 5 minutes, then 7.5% (w/v) sodium carbonate solution 1.2 mL was added to neutralize the reaction mixture. The resulting solutions were incubated at ambient temperature, after which absorbance was recorded at 760 nm using a UV–Visible Spectrophotometer. In the case of the test samples, 0.3 mL of each crude extract (5 mg/mL) was subjected to the same procedure. Total Phenolic content was expressed as gallic acid equivalent (mg/g) using the following equation $Y = 0.001X + 0.0013$, $R^2 = 0.996$ where, y was the absorbance and x was the concentration

Total Flavonoid Content

The total flavonoid content of the extracts were determined using a colorimetric procedure modified from the method described by Zhishen and co-workers[23]. Where Quercetin was employed as the reference compound, and a series of standard solutions (100, 200, 400, 600, 800, and 1000 µg/mL) were prepared. For each determination, Quercetin solutions 0.3 mL was mixed with 5% sodium nitrite ($NaNO_2$) solution 0.06 mL and left to react at ambient temperature for 5 minutes. Thereafter, 10% aluminum chloride ($AlCl_3$) solution 0.06 mL was introduced into the mixture, this was followed by the addition of 0.1 M sodium hydroxide (NaOH) 0.4 mL and finally distilled water 0.5 mL was added to complete the reaction volume. The reaction mixtures were allowed to stand for 30 minutes to ensure full color development, after which the absorbance was measured at a wavelength of 510 nm using a spectrophotometer. For the crude extracts, 0.3 mL of the respective plant extracts (5 mg/mL) solution were treated identically. The flavonoid content was expressed as quercetin equivalent (mg/g) using the following equation $Y = 0.001X + 0.015$, $R^2 = 0.999$ where, y was the absorbance and x was the concentration

Cardiac Glycoside Content

Cardiac glycoside content was determined using digoxin as the reference standard. A series of digoxin solutions at concentrations of 100, 200, 400, 600, 800, and 1000 µg/mL were prepared. To each of the standard solution was added freshly prepared Baljet's reagent 10 mL (comprising 1% picric acid and 10% sodium hydroxide) and allowed to stand for 1 hour to ensure complete color development. The reaction mixture was subsequently diluted with distilled water 20 mL and the absorbance was measured at 495 nm using a UV–Visible spectrophotometer. 10 mL of the plant extract (5 mg/mL) was substituted inplace of digoxin standard and similar treatment as above was followed. the same procedure. Cardiac glycoside content was expressed as digoxin equivalent (mg/g) using the following equation $Y = 0.001X - 0.002$, $R^2 = 0.996$ where, y was the absorbance and x was the concentration c is the concentration

Anti-microbial Assay

The antimicrobial assay was carried out in the Nigerian Army 16 Division laboratory, Yenagoa, Bayelsa State. The test organisms included typed microbial strains (*S. aureus* NCTC8532, *B. subtilis* NCTC1040, *P. aeruginosa* NCTC10662, and *E. coli* NCTC10418) and clinical isolate microbial strains (*S. aureus*, *E. coli*, and *P. aeruginosa*). Antimicrobial susceptibility testing was conducted using strict aseptic techniques throughout the procedure. Both typed strains and clinical bacterial isolates were first subcultured in sterile nutrient broth and incubated at 37°C for 24 hours to obtain active growth. The resulting cultures were standardized by adjusting the turbidity with sterile

normal saline to match the 0.5 McFarland standard, corresponding to approximately 1.0 × 10^8 CFU/mL, in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2025). The agar well diffusion technique, as outlined by CLSI[24], was employed for the antimicrobial assay. Each experiment was conducted in triplicate. After inoculation and introduction of the test samples into the wells, the plates were left undisturbed at room temperature for 30 minutes to permit adequate diffusion of the extracts into the agar medium. Subsequently, the plates were incubated at 37°C for 24 hours, following the method previously reported by Esimone et al.[25]. At the end of the incubation period, the diameters of the inhibition zones were carefully measured in millimeters using a transparent, millimeter-calibrated ruler.

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) of the extracts was determined in accordance with the methods described by El-Mahmood et al[26] and Vinothkumar et al.[27], with slight modifications. Briefly, a series of two-fold serial dilutions of each extract was prepared to obtain concentrations of 500, 250, 125, 62.5, 31.25 and 15.625µg/mL (10µL). Each prepared dilution was inoculated with the respective test bacterial strain under aseptic conditions. The inoculated tubes were then incubated at 37°C for 18 hours. Following incubation, the MIC was defined as the lowest concentration of the extract at which no visible turbidity or microbial growth was observed.

Minimum Bactericidal Concentration (MBC):

For the determination of the minimum bactericidal concentration (MBC), equal volumes of broth cultures that showed no visible growth during the MIC assay were selected. These samples were aseptically transferred and streaked onto sterile Mueller–Hinton agar plates in triplicate. The plates were then incubated at 37°C for 18 hours. The MBC was defined as the lowest concentration of the extract at which no bacterial colony formation was observed on the agar surface, in accordance with the procedure described by Mann et al.[28].

Statistical analysis

Student's t-test was used to ascertain the significance of differences between mean values of the two extracts and the level $p < 0.05$ was considered as the cutoff value or significance. Detailed statistical analyses of the antimicrobial assay data and the susceptibility profiles of all test microorganisms are provided in the Appendix.

RESULTS

The qualitative phytochemical screening of the leaf extract of *B. owariensis* revealed the prescence of various bioactive compounds as summarized in Table 1. below

Table 1: Qualitative phytochemical screening of the leaf extracts of *B. owariensis* leaves

PHYTOCHEMICALS	INFERENCE	
	70%-ETHANOL EXTRACT	N- HEXANE
Tannins	++	-
Terpenoids	-	++
Coumarins	-	+
Saponin	+++	-
Alkaloids	++	++
phlobatannins	++	-

Flavonoids	+++	++
Cardiac glycosides	+++	++
Anthocyanins	+++	-
Gums and muscilage	+++	-
Fixed oil	-	+++
Phenolics	++	+

KEY; - = Not present + = Present in little quantity

++ = Present in high quantity +++ = Present in large quantity

The quantitative phytochemical analysis of *B. owariensis* leaf extracts presented in Fig. 2 below revealed varying concentration of cardiac glycoside, phenol and flavonoids present.

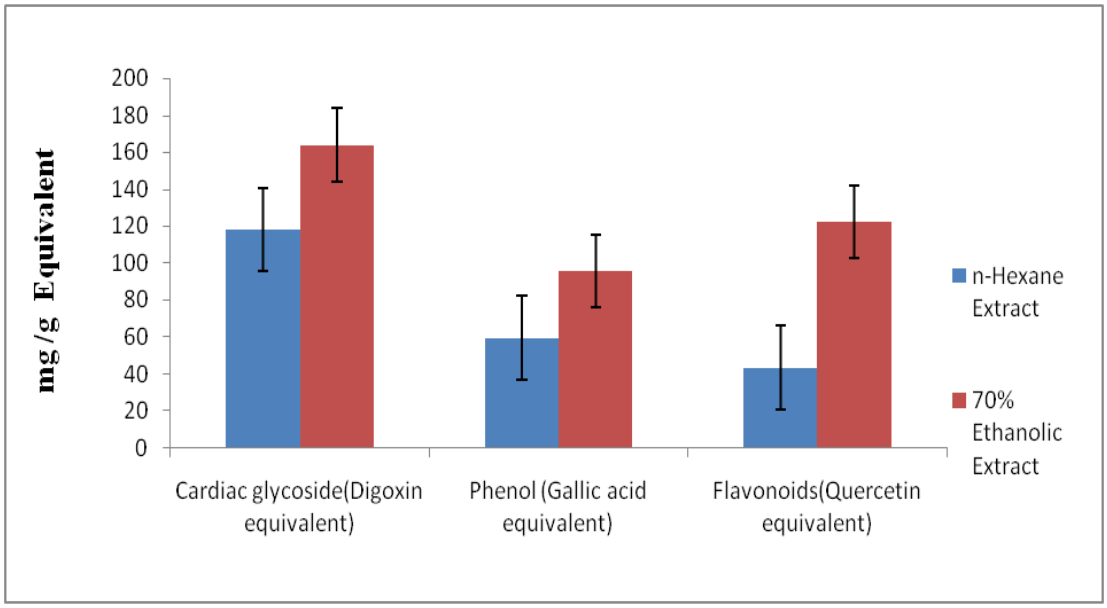

Fig 1: Quantitative phytochemicals screening of the leaf extracts of *B. owariensis*

The Zone of inbhibition demonstrated by *B. owariensis* extracts, presented in Table 2. reveals its potential antimicrobial activity against typed and clinical microbial strains.

Table 2. Anti-microbial activity of the leaf extract of *B. owariensis*

Microorganisms	Mean Zones of Inhibition (mm) and SD			
	E Extract Mean±SD	n-Hex Extract Mean±SD	Cip (5µg/mL)	Control
S. aureus NCTC8532	24.5±0.6	22.3±0.6	35.5±1.2	0
B. subtilis NCTC10400	29.7±0.6	18.6±0.6	35.5±1.2	0
P. aeruginosa NCTC10662	15.3±1.2	26.3± 1,5	35.5±1.2	0

E.coli NCTC10418	31.3±0.6	30.0±1.0	35.5±1.2	0
Clinical Isolates				
S. aureus	20.2±0.6	20.2±0.6	R	0
E.coli	15.3±0.6	15.3±0.6	R	0
P. aeruginosa	23.3±0.6	23.3±0.6	R	0

Keys E: Ethanolic Extract, n-Hex: n-hexane Extract, CIP: Ciprofloxacin; R: Resistant

The MIC and MBC of the ethanolic extract of *B. owariensis* presented in Table 3. indicates its potency against various microorganisms.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the ethanolic extract of *Brillantaisia owariensis*

Microorganisms	Ethanolic extract (µg/mL)							
	500	250	125	62.5	31.25	15.625	MIC	MBC
S. aureus NCTC8532	-	-	-	-	+	+	62.5	125
B. subtilis NCTC10400	-	-	-	-	+	+	62.5	125
P. aeruginosa NCTC10662	-	-	+	+	+	+	62.5	125
E.coli NCTC10418	-	-	-	-	-	+	31.25	62.5
Clinical Isolates								
S. aureus	-	-	-	-	+	+	62.5	125
E.coli	-	-	-	-	-	+	31.25	62.5
P. aeruginosa	-	-	+	+	+	+	62.5	125

Key:

S. aureus = *Staphylococcus aureus.*
subtilis = *Bacillus subtilis*

P. aeruginosa = *Pseudomonas aeruginosa*.
coli = *Escherichia coli*

+ = Microbial growth

B. -= No microbial growth

The MIC and MBC of the n-Hexane extract of *B. owariensis* presented in Table 4. indicates its potency against various microorganisms.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the n-Hexane extract of *Brillantaisia owariensis*

Microorganisms	n-Hexane (µg/mL)							
	500	250	125	62.5	31.25	15.625	MIC	MBC
S. aureus NCTC8532	-	-	-	+	+	+	125	125
B. subtilis NCTC10400	-	-	-	+	+	+	125	250
P. aeruginosa NCTC10662	-	-	-	+	+	+	125	125
E.coli NCTC10418	-	-	-	-	+	+	62.5	125
Clinical Isolates								
S. aureus	-	-	-	-	+	+	62.5	125
E.coli	-	-	-	-	-	+	31.25	62.5
P. aeruginosa	-	-	-	+	+	+	62.5	125

Key: *S. aureus* = *Staphylococcus aureus*. *B. subtilis* = *Bacillus subtilis*

P.aeruginosa = *Pseudomonas aeruginosa*. *E. coli* = *Escherichia coli*

+ = Microbial growth - = No microbial growth

DISCUSSION

The qualitative phytochemical screening result depicted in Table 1, revealed that the plant extracts possess a diverse array of bioactive constituents, including phenolics, saponins, cardiac glycosides, steroids, and gums. These phytochemicals have been widely reported to play significant roles in the management of sickle cell disease (SCD). Specifically, phenolic compounds have been reported to exert their effects through multiple mechanisms, including antioxidant activity, inhibition of hemoglobin S (HbS) polymerization, stabilization of red blood cell (RBC) membranes, anti-inflammatory effects, and enhancement of blood flow. Collectively, these actions contribute to the preservation of erythrocyte integrity, reduction in vaso-occlusive episodes, and prolongation of RBC lifespan as reported by Ochwang'i et al.,[13]. Saponins have also been reported to reduce osmotic fragility of erythrocytes, exhibit anti-inflammatory properties, and modulate pain pathways via opioid receptors and calcium ion (Ca^{2+}) regulation, thereby potentially alleviating chronic pain crises associated with SCD as highlighted by Kouakou et al.,[15]. Steroidal compounds contribute to erythrocyte membrane stabilization and possess anti-inflammatory effects, which may help reduce pain episodes and prevent cellular damage[29]. Additionally, gums are believed to form a protective coating on the RBC surface, thereby shielding the cells from mechanical fragility and oxidative stress, ultimately preventing hemolysis as reported by Chikezie,[30]. the prescence of these phytochemicals suggest that the plant may possess notable anti-inflammatory activity, inhibit hemoglobin S (HbS) polymerization, stabilize red blood cell (RBC) membranes, and enhance blood flow. The quantitative phytochemical analysis result shown in Table 2 further supports the antisickling potential of the plant, as evidenced by the substantial levels of cardiac glycosides (163.4 ± 0.0031mg/g), phenols (98.6 ± 0.0015mg/g), and

flavonoids (125.2 ± 0.0026mg/g) in the polar extract and (118 ± 0.00252mg/g), (62.4 ± 0.001mg/g), and (46.6 ± 0.0026 mg/g) respectively for the n-hexane extract. The phytochemical profile is indicative of strong therapeutic potential, particularly as high phenolic and flavonoid contents are known to confer antioxidant protection, stabilize erythrocyte membranes, and reduce hemolysis. Cardiac glycosides may further contribute by modulating ion transport mechanisms, thereby preventing RBC dehydration and delaying HbS polymerization. The combined presence of these compounds suggests that the plant extract may effectively reduce sickling, protect erythrocytes from lysis, and mitigate inflammatory processes as reported by Ochwang'i et al.,[13]. Furthermore, The antimicrobial evaluation results (Table 2 - 4) demonstrated that the leaf extracts possess broad-spectrum antibacterial activity, the extracts successfully inhibited the growth of several reference typed strains, including *Staphylococcus aureus* NCTC8532, *Bacillus subtilis* NCTC10400, *Pseudomonas aeruginosa* NCTC10662, and *Escherichia coli* NCTC10418, achieving a zone of inhibition equivalent to 60 -70% of that produced by the reference antibiotic, ciprofloxacin. Crucially, the extracts also exhibited potent efficacy against Ciprofloxacin-resistant clinical isolates of *S. aureus*, *E. coli*, and *P. aeruginosa*. This broad-spectrum anti-microbial potential highlights the therapeutic relevance of *B. owariensis*, in sickle cell disease management, particurlarly in preventing infection-triggered vaso-occlusive crises. positioning it as a promising candidate for anti-sickling therapy capable of simultaneously managing secondary bacterial infections in sickle cell disease (SCD) patients. Infections are leading cause of morbidity and mortality in SCD patients, often exacerbated by functional asplenia and frequent hospitalization[31]. By inhibiting key pathogens implicated in osteomyelitis, sepsis, and pulmonary infections, the extracts may mitigate infection-induced inflammation, fever, and hypoxia factors known to precipitate sickling events[32.] Overall, these findings provide scientific validation for the traditional use of *B. owariensis* in mitigating SCD complications. The dual presence of bioactive-antisickling phytochemicals and potent antimicrobial properties positions this plant as a promising therapeutic candidate for sickle cell therapy.

CONCLUSION

The findings of this study demonstrated that *Brillantaisia owariensis* possesses a broad spectrum of bioactive compounds, which includes; phenolics, terpenoids, steroids, alkaloids, coumarins, gums, and lipids. Quantitative analysis further revealed high concentrations of flavonoids, cardiac glycosides, and phenolics, classes of compounds widely associated with antisickling activity. The plant extracts exhibited broad-spectrum antimicrobial activity against pathogens implicated in causing infection triggered crises in SCD, and also efficacious against Ciprofloxacin-resistant microbial strains. these findings provide scientific validation for the traditional use of *B. owariensis* in the management of sickle cell–related complications and highlights its potential for further pharmacological development.

Ethics approval and consent to participate

Human participants or animal model were not used in the study by any of the authors.

- Consent for publication

All authors have read and approved the manuscript and grant their formal consent for its publication in the pharmaceutical journal.

- Availability of data and materials

All datasets generated or analyzed during the current study are fully included within this published article.

- Competing/ conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

Contributor Roles Taxonomy (CRediT)

Ere, Diepreye: Conceptualization, Supervision, Review And Editing, Visualization

Abdulrasheed B. Abdu: Investigation, Formal Analysis.

David T. Tamarabrakemi: Formal Analysis, Methodology

K E. Edoghotu: Investigation, Writing, Data Curation, Software, Visualization.

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