SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: https://doi.org/10.36346/sarjpm.2026.v07i06.001

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Antibacterial Activity and Phytochemical Screening of Blue Pussy Plant Leaf and Flower (*Nelsonia canescens*) against *Escherichia coli and Salmonella sp.*

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Article History: | Received: 20.07.2026 | Accepted: 14.09.2026 | Published: 05.11.2026 |

Abstract: The increasing prevalence of multidrug-resistant bacteria such as *Escherichia coli* and *Salmonella spp*. has heightened the search for novel antimicrobial alternatives. This study explored the phytochemical constituents and antibacterial activity of *Nelsonia canescens*, a plant widely used in traditional medicine. Fresh leaves and flowers were obtained from Tudun Wada Market in Gusau, Zamfara State, Nigeria, and extracted using ethanol and water. Bacterial isolates, collected from the Microbiology Laboratory at Federal University Gusau, were characterized through colonial morphology and verified using standard biochemical techniques. Phytochemical screening identified the presence of alkaloids, saponins, flavonoids, tannins, phenols, and glycosides in both aqueous and ethanolic extracts, while quinones were found only in the ethanolic flower extract. Antibacterial activity was evaluated through agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Ethanol extracts consistently exhibited stronger antibacterial effects compared to aqueous extracts. The maximum inhibition zone (22 mm) was observed against *Escherichia coli* at 200 mg/ml with ethanol leaf and flower extracts, whereas aqueous extracts produced slightly lower activity (20 mm). A statistically significant result ($p = 0.0003$) validated the potency of the extracts.

Keywords: Antibacterial Activity, *Nelsonia Canescens,* Quinones, MIC and MBC.

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INTRODUCTION

The increasing burden of antibiotic-resistant bacteria has formulated into a serious health challenge globally, reducing the efficiency of medication guidelines, and worsening the influence of infectious diseases (Chinemerem *et al*., 2022). Among the most common and clinically important pathogens are Escherichia coli and Salmonella species, both of which are causative agents for severe gastrointestinal and systemic infections across the globe. Multidrug-resistant (MDR) strains of these organisms demonstrate a serious threat, particularly in regions with limited access to effective healthcare, thereby emphasising the urgent need for alternative medical compounds (Alara *et al*., 2024). Medicinal plants have long served as a important source of novel bioactive elements with antimicrobial potential. Their affordability, accessibility, and wide-ranging phytochemical constituents make them promising candidates in the search for new antibacterial agents. Nelsonia canescens, commonly known as the blue pussy plant, is a small herbaceous plant which belongs to the Acanthaceae family. It is widely prevalent across tropical and subtropical regions and has traditionally been used in indigenous medicine for managing wounds, skin infections, fever, and gastrointestinal disorders (Adeleye *et al*., 2021). The therapeutic potential of this plant is associated with its phytochemical components including alkaloids, flavonoids, tannins, and saponins, which are known to

demonstrate antimicrobial, anti-inflammatory, and antioxidant properties (Nwozo *et al*., 2023). Exploring the antibacterial potential of Nelsonia canescens against Escherichia coli and Salmonella spp. is therefore of scientific and public health value. Such studies not only validate traditional medicinal practices but also provide a foundation for the development of novel plant-based therapeutics. This study will assess the antibacterial activity of Nelsonia canescens leaves and flowers and screen their phytochemicals to identify potential new treatments for drug-resistant bacteria.

MATERIALS AND METHODS

Study Area

This study was conducted in Federal University Gusau, Zamfara State, Nigeria. Gusau lies at latitude 12.164°N and longitude 6.667°E, with an estimated population of about 5,833,500. The area is predominantly inhabited by the Hausa and Fulani ethnic groups, while its main economic activities revolve around agriculture and small-scale gold mining.

Collection of *Nelsonia canescens* Leaf and Flower

Nelsonia canescens leaf and flower were obtained from Tudun Wada, Gusau, Zamfara state Nigeria. The obtained plant part (leaf and flower) was authenticated at Federal University Gusau Herbarium, Department of Biological Sciences (Voucher No.: FUG/BIO/HEB/2025/220). Plant part was cleaned with water and dried under shade (to prevent the damage of essential components of the plant) for seven days. After drying, the leaf and flower were grinded into powder and kept in a container for further analysis.

Bacteria Isolate Collection

Bacterial isolates stored in the refrigerator under 4^0C were obtained from Federal University Gusau Microbiology Laboratory.

Sub-Culture of Bacterial Isolates from Stored Isolates

Escherichia coli and *Salmonella sp*. isolates obtained from the microbiology laboratory Federal University Gusau, streaked onto nutrient agar and incubated at 37°C for 24 hours to obtain pure culture (Abubakar *et al.,* 2023).

Preparation of Plant Extracts

The extraction of *Nelsonia canescens* leaf and flower was carried out with the use of ethanol and water as solvents of extraction. The extraction was performed with the use of cold maceration method. 100 g of the grinded leaf was extracted using 1000 mL of each solvent. After 72 hours of constant shaking, the mixtures were filtered. The filtrates were placed in water bath at 42^0C to settle and evaporate to dryness. It was then grinded and stored in sterile containers at 4°C for further use.

Phytochemical Analysis of *Nelsonia Canescens* Extracts

The phytochemical screening of *Nelsonia canescens* extracts was conducted using the slightly modified methods of Daniel *et al.,* (2022).

Standardization of Bacterial Inoculum

To prepare a 1% v/v sulphuric acid solution, 1 ml of concentrated H_2SO_4 was diluted with 99 ml of water and mixed well. In the same way, a 1% v/v barium chloride solution was made by dissolving 0.5 g of dehydrated $BaCl_2$ in 50 ml of distilled water. Then, 0.6 ml of the $BaCl_2$ solution was added to 99.4 ml of the sulphuric acid solution and thoroughly mixed (Abdullahi *et al.,* 2025). A small volume of turbid solution was transferred to screw capped bottle and used for preparing the inoculum.

Antibacterial Susceptibility Testing

The antibacterial efficacy of *Nelsonia canescens* leaf and flower extracts against the bacteria isolate was evaluated using the following methods:

Agar Well Diffusion Method

Mueller-Hinton agar plates were inoculated with standardized bacterial suspensions (0.5 McFarland). Wells were made in the agar with the use of sterile cork borer and filled with plant extracts at concentrations of 25, 50, 100, and 200 mg/ml respectively (Mukesi *et al*., 2019). After incubation at 37°C for 24 hours, the zones of inhibition were measured in millimeters. The Minimum Inhibitory Concentration (MIC) was determined through broth dilution by preparing serial dilutions of the extracts and inoculating them with test isolates. Tubes with no visible turbidity after 24 hours were recorded as the minimum inhibitory concentration (Kebede *et al.,* 2021). The Minimum Bactericidal Concentration (MBC) was determined from MIC-negative tubes and were sub-cultured onto nutrient agar. The MBC was defined as the lowest concentration with absence of bacterial growth.

RESULTS

Table 1: The pytochemical screening revealed the presence of alkaloids, saponins, flavonoids, tannins, and phenols across all extracts. Ethanol extracts additionally contained terpenoids and triterpenes, suggesting a broader phytochemical profile that may contribute to enhanced bioactivity. Table 2 and 3: The extracts demonstrated dose-dependent antibacterial activity against both isolates. Ethanol extracts exhibited higher activity than aqueous extracts, with EFE showing the highest zone of inhibition of 21mm against Salmonella sp. at 50 mg/ml. The activity was statistically significant ($p = 0.0003$). Table 4: The MIC results indicated that EFE had the lowest MIC, inhibiting both isolates at 25 mg/ml, while ALE required 200 mg/ml to inhibit growth. This suggests ethanol flower extract possesses stronger antibacterial constituents. Table 5: Presents the Minimum Bactericidal Concentration of

Nelsonia canescens leaf and flower extracts against the test isolates. The ethanol and aqueous extract completely inhibit Salmonella sp. at 100 mg/ml. the aqueous leaf extract do not have bactericidal concentration on Salmonella sp.

Table 1: Phytochemical Constituents of *Nelsonia canescens* Aqueous and Ethanol Leaf and Flower Extracts

Phytochemical Constituents	Aqueous Leaf	Aqueous Flower	Ethanol Leaf	Ethanol Flower
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Glycosides	+	-	-	+
Resins	-	-	+	-
Flavonoid	+	+	+	+
Terpenoid	-	-	+	+
Triterpenes	-	-	+	+
Steroids	-	-	+	-
Tannins	+	+	+	+
Phenol	+	+	+	+
Quinone	-	-	-	+

Key: + = Positive, - = Negative

Table 2: Zone of Inhibition of *Nelsonia canescens* Aqueous and Ethanol Leaf Extracts against the Isolates

		Concentration (mg/ml)/Zone of Inhibition (mm)					
Isolate	Extract	25	50	100	200	Control	p-value
Salmonella	ALE	0	15	10	18	28	0.0003
sp.	ELE	10	15	15	20		
Escherichia	ALE	10	0	10	15	30	
coli	ELE	10	15	20	22		

Key: ALE = Aqueous Leaf Extract, ELE = Ethanol Leaf Extract
Note: $p < 0.05$ indicates statistically significant results.

Table 3: Zone of Inhibition of *Nelsonia Canescens* Aqueous and Ethanol Flower Extracts against the Isolates

		Concentration (mg/ml)/Zone of Inhibition (mm)					
Isolates	Extract	25	50	100	200	Control	P-value
Salmonella	AFE	10	12	15	20	28	0.0003
sp.	EFE	10	21	20	20		
Escherichia	AFE	10	15	18	20	30	
coli	EFE	10	15	20	22		

Key: AFE = Aqueous Flower Extract, EFE = Ethanol Flower Extract
Note: $p < 0.05$ indicates statistically significant results.

Table 4: The Minimum Inhibitory Concentration (MIC) of *Nelsonia canescens* Leaf and Flower Extracts against *Escherichia coli* and *Salmonella sp*

Concentration (mg/ml)	EFE *Escherichia coli*	EFE *Salmonella sp.*	ALE *Escherichia coli*	ALE *Salmonella sp.*
200	-	-	-	-
100	-	-	+	+
50	+	+	+	+
25	+	+	+	+
12.5	+	+	+	+
6.25	+	+	+	+

Key: + = Presence of growth, - = Inhibition of bacteria, EFE: Ethanol flower extract, ALE: Aqueous leaf extract

Table 5: The Minimum Bactericidal Concentration (MBC) of *Nelsonia canecens* Leaf and Flower Extract against *Escherichia coli* and *Salmonella sp*

Concentration (mg/ml)	EFE *Escherichia coli*	EFE *Salmonella sp.*	ALE *Escherichia coli*	ALE *Salmonella sp.*
200	+	+	+	+
100	-	-	-	+
50	-	+	-	+

Key: + = Presence of growth, - = Inhibition of bacteria, EFE: Ethanol flower extract, ALE: Aqueous leaf extract

DISCUSSION

This study examined the antibacterial activity and phytochemical composition of *Nelsonia canescens* leaf and flower extracts against bacteria isolates of *Escherichia coli* and *Salmonella sp*. the phytochemical screening of *Nelsonia canescens* revealed bioactive compounds including alkaloids, saponins, flavonoids, tannins, phenols, and glycosides in aqueous extracts, except that glycosides were absent in the aqueous flower extract. Ethanolic extracts contained a broader range, with terpenoids, resins, triterpenes, and quinones, the latter detected only in ethanol flower extract. These findings suggest ethanol as a superior solvent for extracting diverse secondary metabolites, particularly non-polar compounds with antimicrobial potential (Mukherjee *et al.,* 2024).

Antibacterial assays showed that both aqueous and ethanolic extracts inhibited *Escherichia coli* and *Salmonella sp.*, though ethanol consistently produced larger inhibition zones. For *Escherichia coli*, ethanol leaf and flower extracts achieved 22 mm zones of inhibition at 200 mg/ml, compared with 15–20 mm in aqueous extracts. Against *Salmonella sp.*, ethanol flower extracts reached 20 mm, similar to aqueous flower extracts. These values, though lower than those of standard antibiotics (28–30 mm), demonstrate meaningful antibacterial effects. The statistical analysis confirmed significant differences ($p = 0.0003$), emphasizing the extracts' efficacy.

The antimicrobial activity is attributable to phytochemicals such as tannins, flavonoids, alkaloids, saponins, phenolics, and quinones, known to disrupt bacterial membranes, interfere with enzymatic functions, and inhibit protein synthesis (Chukwuebuka *et al.,* 2015; Ferdes and Mariana, 2018). The MIC and MBC determinations showed that ethanol extracts had higher inhibitory and bactericidal concentrations than aqueous extracts, highlighting their greater potency. These results align with findings by Wendakoon *et al.,* (2012), who noted that extracts with high MIC and MBC values possess significant antimicrobial potential.

Variations in susceptibility between the two pathogens may reflect differences in outer membrane structure and resistance mechanisms. While both are Gram-negative, *Salmonella sp.* expresses additional virulence factors such as the Vi antigen, which may hinder penetration of phytochemicals and affect sensitivity (Jajere and Sale 2019).

Taken together, *Nelsonia canescens* demonstrated notable antibacterial activity, particularly

in ethanolic extracts, underscoring its therapeutic potential as a natural alternative against multidrug-resistant enteric pathogens.

CONCLUSION

The findings of this study provide substantial scientific support for the traditional use of *Nelsonia canescens* in managing gastrointestinal infections. Phytochemical analysis of the plant's leaf and flower extracts confirmed the presence of diverse bioactive compounds, including alkaloids, flavonoids, tannins, saponins, phenols, glycosides, and quinones, particularly abundant in ethanolic extracts. These metabolites are widely recognized for their antimicrobial activities and are likely responsible for the inhibitory effects observed. Antibacterial evaluations revealed that both aqueous and ethanolic extracts of *Nelsonia canescens* leaves and flowers suppressed the growth of *Escherichia coli* and *Salmonella sp*., with ethanol extracts demonstrating consistently higher efficacy across all tested concentrations. The maximum inhibition zone (22 mm) was achieved with ethanol extracts at 200 mg/ml, while aqueous extracts exhibited comparatively lower, yet significant, activity. Statistical analysis ($p = 0.0003$) confirmed that the antibacterial activity was significant and concentration-dependent. Furthermore, MIC and MBC determinations established both bacteriostatic and bactericidal properties of the extracts, with ethanol preparations showing lower MICs (12.5 - 25 mg/ml) and MBCs (50 - 100 mg/ml). *Salmonella sp*. exhibited greater susceptibility than *Escherichia coli*. Collectively, these findings highlight the pharmacological value of *Nelsonia canescens* and its promise as a source for developing plant-derived antimicrobial agents against resistant enteric bacteria.

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