

Phytochemical Analysis and Antibacterial Efficacy of *Nauclea Latifolia* L (African Peach/Bon Bon Leaf) Leaf Extracts Against Bacterial Isolates - *Salmonella Typhi* & *Escherichia Coli*

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ABSTRACT

Nauclea latifolia Sm, a useful medicinal plant belonging to the Rubiaceae family, is found in the humid tropical rainforest and West and Central Africa. This study aims to investigate the phytochemical constituents and antibacterial efficacy of *Nauclea latifolia* (African Peach/Bon Bon leaf) ethanol leaf extracts against two clinically significant bacterial pathogens: *Salmonella typhi* and *Escherichia coli*. Fresh leaves of *N. latifolia* were collected around the Fendall Campus, University of Liberia, dried and ponded into fine powder, 50g of the powder was macerated in 250mL of ethanol, resulting in a percent yield of 5.4%. After the Qualitative phytochemical screening of the phytoconstituents documented the presence of key bioactive compounds, including saponins, steroids, anthraquinones, flavonoids, alkaloids, tannins, and resins phytochemicals known for their therapeutic and antimicrobial properties. Antibacterial activity was assessed using the agar disk diffusion method, with ciprofloxacin as the standard control. The ethanolic extracts exhibited concentration dependent inhibitory effects on both *S. typhi* and *E. coli*, producing inhibition zones ranging from 16 mm to 22 mm, while ciprofloxacin demonstrated higher inhibition zones of 35 mm and 26 mm, respectively. Although the extract showed moderate antibacterial activity compared to the standard antibiotic, it remained effective and nonresistant within Clinical and Laboratory Standards Institute (CLSI) interpretation thresholds. These findings support the traditional medicinal use of *N. latifolia* and highlight its potential as a complementary agent in managing bacterial infections, especially in settings confronted with rising antimicrobial resistance. Further research is recommended to isolate active constituents, determine minimum inhibitory concentrations, and evaluate potential synergistic interactions with conventional antibiotics.

Keywords: Antibiotic Susceptibility, phytochemical, *E. coli*

INTRODUCTION

Plants have formed the foundation of sophisticated traditional medicine system that have been in existence for thousands of years, not only in Africa, but that tradition herbs are also being used globally to prevent, diagnose, improve, or treat physical and mental illness (Ezeagha, 2023). The World Health Organization (WHO) projected that 80% of the world's population receives their primary treatment from traditional medicine, which is primarily derived from plants (Ezeagha, 2023). Several plants in Africa with promising potentials are used in several modern pharmaceuticals, and drugs are modeled and derived from these bioactive compounds, e.g., aspirin is made with salicylic acid obtained from *Salix alba* bark and, artemisinin from *Artemisia annua* and quinine from the bark of *Cinchona pubescens* are anti-malarial medicines. (Awuchi, 2023). In Montserrado County, Liberia, a total of ninety-two (92) species of medicinal plants belonging to thirty-one (31) families are frequently utilized to make herbal remedies for fifteen (15) different medical conditions. The most common ailments that needed treatment were malaria, goa, typhoid, dysentery, the common cold, piles, and infertility and the most commonly

used plant parts were the leaves, and the Fabaceae family was most commonly mentioned as being used to treat these conditions (Morlia et al, 2025). *Nauclea latifolia*, a member of the Rubiaceae family and native to tropical and subtropical regions of Africa (West and Central Africa), is also referred to as African Peach “Bon Bon Leave” in most part of Liberia. It is a valuable medicinal plant that is frequently used in traditional medicine to treat a variety of illnesses, such as bacterial infections, fever, gastrointestinal issues, and malaria (Balogun, 2016).

MATERIALS AND METHODS

Plant Collection and Identification

The fresh leaves of *N. latifolia* plant were obtained around the Fendall Campus, Upper Montserrado, Liberia. The plant was identified and authenticated by the Biology Department - University of Liberia. The identification was done based on the literature and Atlas of medicinal plants from the Biology Department, University of Liberia.

Plant Preparation

The leaves of *N. Latifolia* were washed with tap water, rinsed with distilled water, and air dry at the T. J. R Faulkner College of Science and Technology laboratory for four weeks at room temperature in a shaded area. The dried plant leaves were pulverized using sterile laboratory Mortar and Pestle to obtain the powder. A Mesh sieve of 0.25 mm was used to sieve the pounded powder to get a fine powder.

The fine powder form weigh 109.5g was taken to the National Reference Laboratory (NRL) of the National Public Health Institute of Liberia (NPHIL) where the antibacterial sensitivity testing and photochemical screening was carried out. Then 50g of the plant powder were macerated successively in 250 ml of ethanol in a beaker at room temperature for 72hours and then the extracts were filtered using a Whitman filter paper, the filtrates were evaporated to dryness at room temperature using a water bath at a temperature below 64°C to remove the solvents used in the extraction. The remaining dry extract weighed and stored in an airtight bottle until further usage.

Percent Yield Concentration (PYC) of Extract

Percent Yield Concentration was determined by dividing the total gram of crude extract that constitute 2.70g by the total gram of powder extract that constitute 50g and multiplies by 100%.

$$\text{PYC} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100\%$$

Theoretical yield

Phytochemical Screening

Phytochemical screening of major chemical constituents was carried out using standard qualitative methods similar to what was used by Muhammad et al. (2022). The constituents tested were anthraquinones, alkaloids, glycosides, flavonoids, resins, saponins, steroids and tannins.

Test for anthraquinones

4mL of each extract was placed in a test tube, 4mL of 100% ammonium solution was added. A pink violet or red coloration in the ammoniac layer in each test tube indicated the presence of anthraquinones (Muhammad et al. 2022).

Test for alkaloids

3mL of extract were introduced into the different test tubes, 1mL of 1% of hydrochloric acid (HCl) was added. Then few drops of Mayer's reagent will be added. The Presence of green color or white precipitate indicates the presence of alkaloids (Muhammad et al. 2022).

Test for flavonoids

2mL of extract were pulled into the test tubes, 3-4 drops of NaOH, then add 5mL of dilute HCl, intense yellow disappeared upon acidification indicating the presence of flavonoids (Vijayalakshmi, R. R. 2018).

Test for test resins

5mL of each extract, 5mL of copper acetate solution were added and shaken vigorously, and then allowed to separate. A reddish- brown precipitate indicated the presence of resins (Muhammad et al. 2022).

Test for saponins

0.5 milliliters of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of foam confirmed the presence of saponins (Dubale et al., 2023).

Test for steroids

1mL of crude extract, 1mL of concentrated tetraoxosulphate (IV) acid (H₂SO₄) were added. A reddish coloration indicated the presence of steroids (Vijayalakshmi, R. R. 2018).

Test for tannins.

1mL of each extract, 2 drops of 5% of ferric chloride (FeCl₃) was added. A dirty green precipitate indicated the presence of tannins. (Mohammed et al., 2021).

Bacterial Isolate

The two bacterial isolate tested (*S. typhi* and *E. coli*) for this investigation were sourced from stock cultures at the National Reference Laboratory (NRL) of the National Public Health Institute of Liberia (NPHIL). The viability of the test organisms was established by their growth on Tryptic Soye Agar.

Antimicrobial Sensitivity

Antimicrobial activities of the ethanolic extract of *N. latifolia* were determined by using disk diffusion method for antibacterial activity. Two separate dishes of Mueller Hinton Agar were prepared for both *S. typhi* and *E. coli*, respectively, and swabbed with single colony of *S. typhi* and *E. coli* over the entire plates.

Five (5) minutes after the surface was dried, the cipro simple and extract was dropped on the test organism using a cotton disk. The plates were placed in an incubator at 37°C for 24hrs. Each disk's zone of inhibition was observed and measured in millimeters from one edge to the other.

RESULT

Percent yield concentration of *N. latifolia* leaf extracts

Plant	Weight of plant powder macerated (g)	Crude Extract Weight (g)	% yield (%)
<i>N. latifolia</i>	50.0	2.70	5.4

A total of 50g powdered *Nauclea latifolia* leaf was macerated in 250mL of ethanol, a crude extract weighing 2.70g was obtained, therefore produced 5.4% yield, indicating a moderate concentration of extractable phytochemicals in the plant material.

The qualitative phytochemical screening of *N. latifolia* leaf extracts

Phytochemical Analysis							
Fraction	Tannins	Saponins	Steroids	Anthraquinones	Flavonoid	Alkaloid	Resins
Ethanol	+	+	+	+	+	+	+
Aqueous	+	+	+	+	+	+	+

Key: + present, - absent

Phytochemical screening of *N. latifolia* leaf extract. The phytochemical screening of *N. latifolia* leave extract constituents present in crude ethanol extract and aqueous solution, were Anthraquinone, Steroids, Saponins, Flavonoids, flavonoids, Alkaloid and Tennis.

Antimicrobial activity of the ethanolic leave extract of *N. latifolia* against *E. coli*

Ethanol Extract	Ethanol Extract			Aqueous Extract		
Concentration (mg/mL)	0.5	1.0	1.5	0.5	1.0	1.5
Zone of Inhibition (mm)	17	20	22	16	17	20
Control (Cipro)	26			26		

Key: 00 = No zone of inhibition.

N. latifolia crude leaf extracts antibacterial activity for both ethanol and aqueous extracts. According to the antibacterial activity assay, ciprofloxacin has a wider diameter of 26 mm, followed by 1.5g *N. latifolia* (22 mm) and 1.0g *N. latifolia* (20 mm), which both clearly inhibited *E. coli*. The aqueous leave extract inhibited the bacterial isolate (*E. coli*), 1.5g *N. latifolia* (20 mm) has a wider diameter after ciprofloxacin (26mm) and 1.0g *N. latifolia* (20 mm) followed next, activity peaked at 1.5g/mL (22mm) and then declined at 050g/mL (17mm), indicating a moderate response.

Antimicrobial activity of the ethanolic leave extract of *N. latifolia* against *S. typhi*

Ethanol Extract	Ethanol Extract			Aqueous Extract		
Concentration (mg/mL)	0.5	1.0	1.5	0.5	1.0	1.5
Zone of Inhibition (mm)	18	19	22	16	19	20
Control (Cipro)	35			35		

Key: 00 = No zone of inhibition.

The antibacterial activity assay indicated that the various disk inserted on the *S.typhi* from table 4, ciprofloxacin has wider zone of inhibition of 35mm, followed by the 1.5g *N.latifolia* , and then by 1.0g and at last 0.5g *N. latifolia* for the ethanol extract. The aqueous extract of the antibacterial activity assay indicated that 1.5g *N.latifolia* (20mm) has the wider zone of inhibition after ciprofloxacin which is 35mm, then follow by 1.0g (19mm) and at last 0.5g *N. latifolia* (16mm).

The antimicrobial sensitivity test for the ethanolic extract of *N. latifolia* containing *E.coli*

Extract/CLSI	Ethanol Extract			Aqueous Extract			Ciprofloxacin
Concentration (mg/mL)	0.5	1.0	1.5	0.5	1.0	1.5	
Resistant (≤15mm)							
Intermediate (16-20mm)	+	+		+	+	+	
Susceptible (≥21mm)			+				+

Source: clinical laboratory standard institute (CLSI) zone diameter interpretive standard chat.

Table results demonstrated that *E. coli* were susceptible to both 1.5 g and cipro and intermediate at 0.5 g and 1.0 g but not resistant to any concentration of plant extract or pharmaceutical drugs.

The antimicrobial sensitivity test for the ethanolic extract of *N. latifolia* containing *S. typhi*.

Extract/CLSI	Ethanol Extract			Aqueous Extract			Ciprofloxacin
	0.5	1.0	1.5	0.5	1.0	1.5	
Concentration (mg/mL)							
Resistant (≤15mm)							
Intermediate (16-20mm)	+	+		+	+	+	
Susceptible (≥21mm)			+				+

Source: clinical laboratory standard institute (CLSI) zone diameter interpretive standard chart.

Table results showed *S.typhi* was susceptible to both 1.0 g and cipro and intermediate at 0.5 g and 1.5 g but not resistant to any concentration of plant extract or pharmaceutical drugs.

DISCUSSION

The The phytochemical screening of *N. latifolia* or Bonbon Leaf detected the presence of, Saponin, Steroids, Anthraquinones, Flavonoids, Alkaloid, Tannins and Resins as phytochemical constituents that play therapeutic roles that contributed immensely in their antibacterial activities against *E.coli* and *S.typhi* by inhibiting their growth. This result is similar to the findings of (Muhammad et al., 2020). This result also supports the findings of Malami et al. (2019), who found that extracts from the roots, stem, bark, and leaves showed pharmacological activity supporting some of the ethnomedical uses. Furthermore, bioactive compounds have been identified, characterized, and used for antimalarial applications. These compounds account for a variety of demonstrated actions.

The Clinical and Laboratory Standards Institute states that the diameter of a zone of inhibition in a disc with a 5µg concentration of ciprofloxacin falls into three categories: resistant (≤15 mm), intermediate (16–20 mm), and susceptible (≥21 mm). Ciprofloxacin, and *N. latifolia* 1.5g in table-5, 0.5g and 1.5g in table 6 are susceptible to this standard and the remaining concentrations of *N. latifolia* at intermediate according to the tables. The results of the antibacterial tests indicate that the aqueous extracts and ethanol extracts inhibited *E. coli* and *S. Typhi*, although the degree of inhibition varied according to the concentration. The ethanol extract showed larger inhibition zones (16mm–22mm) then the aqueous extracts (16mm–20mm), indicating that the ethanolic extract had relatively higher antibacterial potency against both *S. typhi* and *E. coli* as compare to the aqueous extract. The results are similar to Enabulele et al. (2017), which verify that the ethanol extracts showed greater zones of inhibition against all test species at different concentration. However, *N. latifolia* extracts showed less inhibition when compared to the standard antibiotic ciprofloxacin, which had zones of inhibition of 26 and 35 mm in *E. coli* and *S. typhi* dishes respectively. This suggests that while *N. latifolia* has antibacterial qualities, its activity is moderate and concentration dependent.

Reports by Enabulele et al. (2017), [11] and [9] have also suggested the efficacy of alcohol-based extracts against bacteria more than water-based extracts. The crude extract value of *N. latifolia* (2.70g) divided by the powder extract value of *N. latifolia* (50.0g) multiplied by 100% yielded the percentage yield concentration of *N. latifolia* leaf extract 5.4%.

CONCLUSION

The results of this study revealed that a rich profile of bioactive phytochemicals, such as saponins, steroids, anthraquinones, flavonoids, alkaloids, tannins, and resins, are present in the ethanolic leaf extract of *Nauclea latifolia*. The antibacterial effects against *Salmonella typhi* and *Escherichia coli* were probably caused by these substances, which are well known for their medicinal and antimicrobial qualities. Ethanol is a useful solvent for extracting pharmacologically active components from the plant, as evidenced by the extraction procedure moderate yield of 5.4%.

The ethanolic extract showed wider inhibitory effects on both *E. coli* and *S. typhi*, as compare to the aqueous extracts with inhibition zones ranging from 16 to 20 mm, according to the antimicrobial evaluation. According to Clinical and Laboratory Standards Institute (CLSI) interpretation standards, the extract consistently showed moderate antibacterial activity, despite the degree of inhibition varying with concentration. While ciprofloxacin produced higher inhibition zones, the plant extract still showed meaningful antibacterial potential, indicating that *N. latifolia* possesses moderate, concentration dependent activity. This suggests that the ethanolic and aqueous leaf extract of *Nauclea latifolia* has important phytochemical and antibacterial qualities, supporting its traditional use in the treatment of infectious diseases, may not serve as a direct replacement for conventional antibiotics, it holds promise as a complementary therapeutic agent, especially in the development of plant based antimicrobials or in regions with increasing antibiotic resistance. To improve knowledge of its pharmacological potential and maximize its therapeutic applications, more research involving fractionation, active compound isolation, toxicity evaluation, and comparison with other extraction solvents is advised.

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