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Phytochemical profile of *Nauclea latifolia* leaf extract: a quantitative and qualitative evaluation for anti-inflammatory and antidiabetic potential

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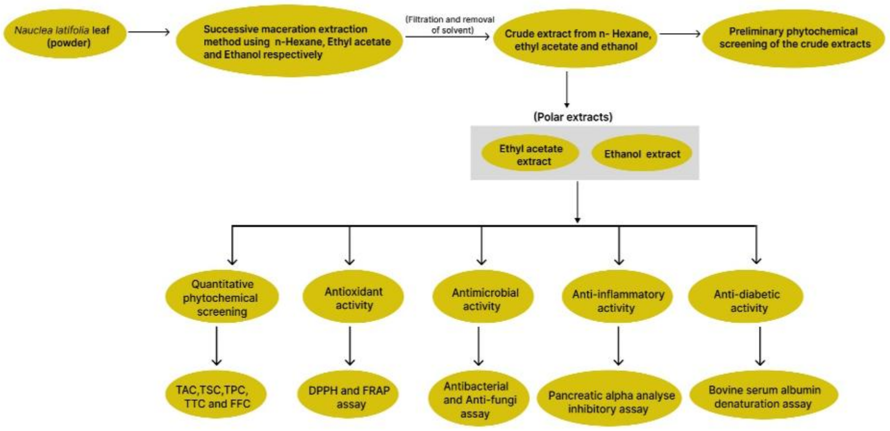
Abstract

Antioxidant agents are essential for the body because of their ability to scavenge free radicals. Medicinal plants contain phytochemicals that act as antioxidants. In this study, phytochemicals, antibacterial, antioxidant, antidiabetic, and anti-inflammatory activities of leaf extracts from *Nauclea latifolia* were evaluated. Secondary metabolites, including saponins, alkaloids, flavonoids, tannins, coumarin, steroids, terpenoids, cardiac glycosides, glycosides, quinones, and polyphenols, were present. The ethanolic extract had the highest quantity of polyphenols (5.6±0.1 mg/g GAE), flavonoids (53.4±0.1 mg/g QE), tannins (3.8±0.1 mg/g TAE), alkaloids (30.3±0.1 mg/g), and saponins (48.10 mg/g). Intense antimicrobial action was observed against the selected pathogenic species. The ethanolic extract exhibited exceptional antioxidant activity, indicated by its low IC_{50} value of 2.11 mg/mL and strong reducing power. More importantly, the ethyl acetate extracts also markedly inhibited pancreatic α-amylase (IC_{50}=2.19 mg/mL) and bovine serum albumin (IC_{50}=2.52 mg/mL). These findings demonstrate that *Nauclea latifolia* is a rich source of bioactive compounds with significant medicinal potential, including antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties. These attributes highlight its suitability for various therapeutic applications.



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Highlights

- Qualitative and quantitative constituents of *Nauclea latifolia* leaf extracts assessed.
- Saponins, alkaloids, flavonoids, and terpenoids were found by phytochemical screening.
- Secondary metabolites have antimicrobial activity against selected pathogenic species.
- The ethanolic extract showed significant antioxidant potential.
- Antioxidant agents play a crucial role in neutralizing free radicals.

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1. Introduction

Medicinal plants are rich in bioactive compounds, which have been utilized for centuries in traditional medicine (Marrelli, 2021). According to the World Health Organization, approximately 80% of people in underdeveloped nations rely on these plants for their primary healthcare needs (Truong *et al.,* 2019). Furthermore, these plants serve as a vital source of antioxidants, protecting against the onset of degenerative diseases and mitigating the harmful effects of free radicals (Rahman *et al.,* 2022). The phytochemical composition of medicinal plants, particularly their antioxidant activity, significantly influences their biological functions. However, variations in phytochemical content can arise due to factors such as climate, harvest time, plant genotype, and botanical origin (Dhalaria *et al.,* 2020; Ghimire *et al.,* 2021). Oxidative stress, which occurs when the body's defenses against free radical damage become imbalanced, has been linked to several human diseases, including diabetes, Parkinson's disease, cancer, and Alzheimer's disease (Lengai *et al.,* 2020; Sharifi-Rad *et al.,* 2020). Natural antioxidant compounds, such as flavonoids, phenolics, and tannins, play a crucial role in combating oxidative stress by neutralizing reactive oxygen species (ROS) and providing protection against related diseases. For instance, inadequate antioxidant intake has been associated with diabetes mellitus, a condition characterized by persistent hyperglycemia. This disease arises when the body either fails to produce sufficient insulin (insulin resistance) or cannot effectively utilize insulin (insulin secretion insufficiency) (Guo *et al.,* 2022; Lengai *et al.,* 2020; Rudrapal *et al.,* 2022). Among medicinal plants, *Nauclea latifolia*, a member of the Rubiaceae family, is noteworthy for its traditional uses and diverse applications (Haudecoeur *et al.,* 2017). Native to tropical Africa and Asia, it is known by various local names in Nigeria, such as egbesi, tafashiya, and ubulu inu (Arise *et al.,* 2012; Galicia-García *et al.,* 2020). This plant, characterized by its spherical, red fruiting structures with adhesive stamens and distinctive floral morphology, is a perennial, evergreen shrub or tree capable of reaching a height of up to 20 meters. It thrives in the moist tropical rainforest zones and savannah woodlands of West and Central Africa (Ajiboye *et al.,* 2019).

Given its extensive traditional application (Boucherle *et al.*, 2016; Haudecoeur *et al.*, 2017), this study aims to explore the chemical profile of *Nauclea latifolia* leaf extracts and investigate their potential antibacterial, antioxidant, anti-inflammatory, and anti-diabetic properties.

2. Experimental

2.1. Collection of plant samples

The *Nauclea latifolia* leaves were collected from the Ibapon hamlet in Ogbomosho, Oyo State, Nigeria, located at coordinates 7° 54' 0" N and 4° 5' 0" E, along the Odo-Oba area. The leaves were identified by Professor A.T.J. Ogunkunle of the Ladoke Akintola University of Technology Ogbomosho's Pure and Applied Biology Department and assigned the Botanical Identification Registration Number No. LHO 392,581. Distilled water was used to wash the plant's leaves to remove contaminants. They were then cut into smaller pieces and allowed to dry in the air for forty days. The dried leaf material was pulverized and kept in an airtight container for further investigation.

2.2. Extraction of plant materials

One thousand two hundred grams (1200 g) of finely crushed plant material was first soaked in n-hexane for seven days. After that, ethyl acetate and ethanol were added to the same extract successively until no more extraction was visible. Following the filtration and concentration of each solvent extract using a rotary evaporator, the weights of the final n-hexane, ethyl acetate, and ethanol extracts were then noted and quantified to determine the extraction yield (**Eq. 1**).

$$\text{Yield } \% = \frac{M1}{M2} \times 100 \quad (1)$$

where, yield % = yield in percentage; M_1= mass of dry crude extract (g); M_2 = mass of pulverized plant materials (g).

2.3. Preliminary phytochemical screening

The phytochemical components of the three crude extracts of *Nauclea latifolia* leave obtained were evaluated using a standardized protocol by Adepoju *et al.* (2023). Saponins, alkaloids, flavonoids, tannins, coumarin, steroids, terpenoids, cardiac glycosides, glycosides, quinones, anthraquinone, anthocyanin, and phenol were among the phytochemicals investigated.

2.3.1. Quantification of total alkaloid content

Five grams (5 g) of the sample were weighed into a 250 mL beaker, and 200 mL of 10% acetic acid in ethanol was added. The beaker was then covered and allowed to stand for 4 h. This was filtered, and the extract was concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The entire solution was allowed to settle, and the precipitate was collected and washed with dilute ammonium hydroxide, then filtered. The residue is the alkaloid, which was dried and weighed (Shaikh and Patil, 2020).

2.3.2. Quantification of total saponin content

The method used was that of Van Tan (2018). The samples were ground, and 20 g of each was placed into a conical flask. Then, 100 mL of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55 °C. The mixture was filtered, and the residue was re-extracted with an additional 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over a water bath at about 90 °C. The concentrate was transferred into a 250 mL separatory funnel, and 20 mL of diethyl ether was added, followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of a 5% aqueous sodium chloride solution. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the Saponin content was calculated as a percentage.

2.3.3. Quantification of total phenolic content

The total phenolics in plant extracts were determined by spectrophotometric analysis utilizing the Folin-Ciocalteu test method (Obadoni and Ochuko, 2002). A reaction mixture was prepared by combining 1 mL of extract, 1 mL of Folin-Ciocalteu reagent, and 9 mL of distilled water in a 25 mL volumetric flask. Five minutes later, 10 mL of a 7% sodium carbonate solution was added. Gallic acid standards (20-100 µg/mL) were treated in the same manner as the samples. A UV-Vis spectrophotometer was used to measure the absorbance of the test and standard solutions at 550 nm following a 90-minute incubation period at room temperature. The total phenol concentration was determined in milligrams of GAE per gram of extract.

2.3.4. Quantification of total tannin content

The Folin-Ciocalteu method (Siddiqui *et al.,* 2017) was used to determine the tannin concentration. 0.1 mL of the sample extract, 7.5 mL of distilled water, 0.5 mL of the Folin-Ciocalteu reagent, and 1 mL of 35% Na_2CO_3 were added to a volumetric flask (10 mL). After shaking, the mixture was left for thirty minutes. Gallic acid standard solutions (20-100 µg/mL) undergo a similar process. A UV-Vis spectrophotometer measured the absorbance at 725 nm. The amount of tannin present in each gram of extract was determined in milligrams of gallic acid equivalent (GAE).

2.3.5. Quantification of total flavonoid content

The aluminum chloride calorimetric approach was used to determine the total flavonoid concentration (Rao, 2016). 1 mL of extract and 4 mL of distilled water were combined to create a solution in a 10 mL volumetric flask. After five minutes, 0.30 mL of treated 5% sodium nitrite and 0.3 mL of 10% aluminum chloride were added. After five more minutes, 2 mL of 1 mol/L sodium hydroxide was added, and the volume was adjusted to 10 mL with distilled water. A set of reference standard solutions for Quercetin (20, 40, 60, 80 and 100 µg/mL) was prepared in the same manner as described earlier. The absorbance of test and standard solutions was determined against the reagent blank at 510 nm with a UV-Vis spectrophotometer.

2.4. Analysis of antimicrobial activity

2.4.1. Evaluation of antibacterial activity

According to the National Committee for Clinical Laboratory Standards, the ethyl acetate and ethanol extracts of *Nauclea latifolia* leaves were tested for their ability to inhibit the growth of gram-positive and gram-negative bacteria, including *Escherichia coli* ATCC 25922*, Salmonella typhi* ATCC 6539*, Klebsiella oxytoca* ATCC 700324*, Staphylococcus aureus* ATCC 25922 and *Proteus vulgaris* ATCC 6380*,* using the well diffusion method. The microbiology department of the BOWEN University Teaching Hospital in Ogbomosho, Oyo State, Nigeria, provided clinical specimens from which all pathogens were isolated. The test organisms were developed separately by subculturing the pure isolates in nutrient agar and incubating them for bacteria for 24 h at 37 °C. Ethanol and ethyl acetate were used as the negative control, while gentamicin and ketoconazole were utilized as the positive control. A stock solution of 1 mg/mL of each crude extract was prepared from which concentrations of 25, 50, 100, and 150 µg/mL were prepared for both extracts. The sensitivity test used the absence of development on or around the plate as a benchmark. Prior to observation and measurement of the zone of inhibition in milliliters, the plates were incubated for twenty-four hours at 37 degrees Celsius.

2.4.2. Evaluation of antifungal activity

The antifungal effects of ethanolic and ethyl acetate extracts were investigated using the mycelia growth inhibition test (Harathi *et al.,* 2017). Extracts were put into potato dextrose agar (PDA) at a concentration of 25 g/mL. A 6 mm agar plug containing 48-hour-old cultures of *Aspergillus niger* ATCC 16404*, Aspergillus fumigatus* ATCC 46645*, Fusarium solani* ATCC 36031*, Penicillium novetatum* ATCC 10431 and *Aspergillus flavus* ATCC 9643 was used to inoculate the PDA plates used in the core portion of this investigation. In the control plates, there were empty nanoparticles. All the plates were incubated at 28 °C for 72 h. After measuring the radial fungal growth on each plate, the following method was applied to calculate the percentage growth inhibitions using **Eq. 2**.

$$\text{percentage growth inhibitions} = \frac{Dcontrol - Dtest}{Dcontrol} \times 100 \quad (2)$$

where, D is the diameter of fungal growth on the PDA plates.

2.5. Analysis of antioxidant activity

2.5.1. Determination of DPPH free radical scavenging activity

The free radical scavenging and antioxidant activities of *Nauclea latifolia* leaves extracts against the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) were evaluated (Synytsya *et al.,* 2017). A stock solution of 35 µg/mL of each crude extract was prepared from which concentrations of 0.5, 2.0 and 3.5 µg/mL were obtained using methanolic solution of DPPH for both extracts. Using vitamin C as a reference material, the absorbance at 517 nm was determined using a spectrophotometer following a 30-minute incubation period at room temperature and in the dark. The percentage inhibition was calculated for each concentration of extracts and standard using **Eq. 3**.

$$\%\ \text{Inhibition} = \left(\frac{Ab - As}{Ab}\right) \times 100 \quad (3)$$

A represents the absorbance of the *Nauclea latifolia* leaf extracts at different doses, and Ab represents the absorbance of the blank solution. The dose-response curve was plotted, and then the extracts and the standard's IC_{50} values were determined.

2.5.2. Ferric reducing antioxidant power assay (FRAP)

With a few modifications, the FRAP evaluation guidelines (Jemli *et al.,* 2016) were adhered to. A 300 nm acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL of acetic acid) with a pH of 3.6 and a ten millimolar solution of 2,4,6-tripyridyl-s-triazine were included in the stock solutions. The new working solution was prepared by mixing 25 mL of $FeCl_3.6H_2O$ solution, 2.5 mL of TPTZ solution, and acetate buffer. After mixing the plant extract sample (50 µL, 1 mg/mL) with the FRAP solution, the mixture was left to react for five minutes without light. Then, at 593 nm, the colorful material ferrous tripyridyltriazine complex was detected. Milligrams of Trolox equivalents per gram of sample were utilized to assess the outcomes.

2.6. Bovine serum albumin assay (BSA)

The anti-inflammatory capabilities of the crude plant extracts were assessed using an altered version of the BSA test (Williams *et al.,* 2008). BSA solution (0.4%, w/v) was prepared in Tris Buffered Saline by dissolving one tablet in 15 mL of deionized water to make 0.05 mg of Tris and 0.15 mg of sodium chloride, which resulted in a pH of 7.6 at 25 °C. Then, glacial acetic acid lowers the pH to 6.4. A stock solution of each plant extract was made in methanol at a concentration of 50 µg/mL, or 0.005%, w/v. The equivalent aliquots of 5.0, 10, and 20 µL, which represented concentrations of 0.25, 0.50, and 1.00 µg/mL of the stock solutions, were placed into test tubes containing 1 mL of 0.4%, w/v BSA buffer solution. Methanol served as negative control and aspirin as the positive control. The solutions were then allowed to cool for 20 min in a lab environment after being heated for 10 min at 72 °C in a water bath. The turbidity (or degree of protein precipitation) of the solutions was measured at 660 nm using a Hach Spectrophotometer and an air blank. The mean absorbance values were recorded after the studies were conducted twice. The percentage inhibition of precipitation (protein denaturation) relative to the negative control was computed using Eq. 4.

$$\% \text{ Anti-Denaturation Activity} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100 \quad (4)$$

where, Anti-Denaturation Activity = % Inhibition of Protein Denaturation = % Anti-inflammatory Activity.

2.7. Pancreatic α-amylase inhibitory assay

The study's standard operating procedures underwent minor modifications (Hansawasdi *et al.,* 2000). 2 mg of starch azure suspension was made in 0.2 mL of 0.5 mol/L Tris-HCl buffer (pH 6.9) containing 0.01 mol/L $CaCl_2$ (substrate solution). A 5-minute preincubation at 37 °C was performed on the substrate solution tubes. Five minutes later, it started to bubble. An extract sample was dissolved in DMSO to produce varying extract concentrations. After that, 0.2 mL of a specific plant extract concentration was added to the substrate solution tube. The tube containing the plant extract and substrate solution also received 0.1 mL of swine pancreatic amylase in Tris-HCl buffer (2 units/mL). For ten minutes, the reaction was performed at 37 °C. Each tube was filled with 0.5 mL of 50% acetic acid to halt the process. The reaction mixture was centrifuged for five minutes at 4 °C and 3000 rpm. Using a spectrophotometer, the absorbance of the resultant supernatant at 595 nm was determined. The standard prescription was ascorbic acid, a well-known inhibitor of α-amylase. Three runs of the trials were conducted. The percentage pancreatic α-amylase inhibitory activity using Eq. 5.

$$\text{Percentage pancreatic α-amylase inhibitory activity} = \frac{[(Ac+)-(Ac-)]-[(As-Ab)]}{[(Ac+)-(Ac-)]} \times 100 \quad (5)$$

where, Ac+, Ac, As and Ab are the absorbance of a test sample (with enzyme), a blank (a test sample without enzyme) and 100% enzyme activity (only solvent with enzyme) respectively.

3. Results and discussion

3.1. Extraction yield

The percentage yield of leaf extracts from *Nauclea latifolia* as depicted in (Table 1), evaluates the extraction efficiency and indicates the quantity of target chemical substances obtained (Nortjie *et al.,* 2022). Less polar compounds are attracted by the nonpolar nature of n-hexane, which yields 0.57 percent from 6.38 g. In contrast, somewhat polar ethyl acetate yields 0.89 percent from 10.69 g, while polar ethanol yields 0.86 percent from 10.35 g. The polarity of the solvents affects the selective extraction process, lipids can be extracted using nonpolar solvents like n-hexane, whereas phenolics and flavonoids can be extracted using polar solvents like ethyl acetate and ethanol (Nawaz *et al.,* 2020). The observed yields agreed with the solvent preferences, highlighting the significance of solvent selection in terms of maximizing extraction procedures to extract particular plant components (Nawaz *et al.,* 2020; Truong *et al.,* 2019).

Table 1. Extraction yield of the plant materials.

Solvent extracts	n-hexane	Ethyl acetate	Ethanol
Solvent yield (g)	6.8±0.0	10.7±0.1	10.3±0.0
Percentage yield	0.6±0.0	0.89±0.0	0.86±0.0

Source: Elaborated by the authors.

3.2. Preliminary phytochemicals screening

The phytochemical composition of *Nauclea latifolia* leaf extracts is presented in Table 2, highlighting the variations in extracted components based on the solvent used. The extract from n-hexane shows a notable absence of certain phytochemicals, such as flavonoids and alkaloids, which are not detected in this extract. On the other hand, a wide range of phytochemicals, such as terpenoids, cardiac glycosides, quinones, tannins, coumarins, flavonoids, alkaloids, and saponins, are revealed in ethyl acetate and ethanol extracts. These chemical compounds are present in ethanol and ethyl acetate extracts because of their moderate to high polarity, which facilitates a more thorough extraction of a wide variety of bioactive chemicals (Amador-Luna *et al.,* 2019). Moreover, the continuous absence of glycosides, anthraquinones, and anthocyanins in both extracts suggests either a limited availability of these constituents in *Nauclea latifolia* leaves or their poor solubility in ethyl acetate and ethanol. This finding aligns with research studied previously (Adepoju *et al.,* 2024; Iheagwam *et al.,* 2020), which reported alkaloids, saponins, tannins, flavonoids, anthocyanin, quinones, cardiac glycosides, and glycosides in *Nauclea latifolia* leaf and stem bark extracts respectively. This emphasizes how crucial the choice of solvent is since it significantly impacts the phytochemical profile and offers helpful guidance on how to improve *Nauclea latifolia* therapeutic potential.

Table 2. Phytochemical composition of extracts.

Phytoconstituents	n-Hexane extract	Ethyl acetate extract	Ethanol extract
Saponins	–	+	+
Alkaloids	–	+	+
Flavonoids	–	+	+
Tannins	–	+	+
Coumarin	–	+	+
Steroids	–	+	+
Terpenoids	–	+	+
Cardiac Glycosides	–	+	+
Glycosides	–	–	–
Quinones	–	+	+
Anthraquinone	–	–	–
Phenol	–	+	+

Note: + represent (present) and – represent (absent)
Source: Elaborated by the authors.

3.3. Quantitative phytochemicals analysis

The quantitative phytochemical analysis of *Nauclea latifolia* leaf extracts using ethanol and ethyl acetate as solvents reveals significant variations in their bioactive compound composition (Fig. 1). The ethanol extract demonstrated higher levels of phenolics 5.56 mg/g GAE (milligrams of Gallic Acid Equivalents per gram) compared to the ethyl acetate extract (3.93 mg/g GAE). Phenolic compounds, known for their potent antioxidant activity, play a crucial role in scavenging free radicals (Platzer *et al.,* 2024). Ethanol, being a polar solvent, is more effective in extracting these compounds due to its ability to solubilize both polar and nonpolar molecules (Alara *et al.,* 2021). Similarly, flavonoid content was notably higher in the ethanol extract 53.56 mg/g QE (milligrams of Quercetin Equivalents per gram) than in the ethyl acetate extract (34.69 mg/gQE). Flavonoids are recognized for their antioxidant and anti-inflammatory properties, and their increased concentration in the ethanol extract can be attributed to ethanol polarity, which facilitates better extraction (Chaves *et al.,* 2020; Ullah *et al.,* 2021). Tannins, with antibacterial and antioxidant properties, were also more abundant in the ethanol extract 3.81 mg/g TAE (milligrams of Tannic Acid Equivalents per gram) than in the ethyl acetate extract (1.31 mg/g TAE). The efficiency of ethanol in dissolving and extracting tannins from plant matrices underscores its utility as a solvent (Altemim *et al.,* 2017; Cano *et al.,* 2021). Alkaloid concentrations followed a similar trend, with 30.30 mg/g in the ethanol extract and 26.5 mg/g in the ethyl acetate extract. Alkaloids exhibit diverse pharmacological activities, including anti-inflammatory and analgesic effects (Heinrich *et al.,* 2021; Kurek *et al.,* 2019). Saponins, known for their antibacterial and anticancer properties, were markedly higher in the ethanol extract (48.10 mg/g) compared to the ethyl acetate extract (1.0 mg/g). This significant difference highlights the role of solvent polarity in extracting these polar bioactive components (Jayaraman *et al.,* 2023; Kumar *et al.,* 2023). Overall, the findings emphasize that the solvent used significantly influences the yield and diversity of secondary metabolites. Ethanol, due to its polar nature, extracted higher concentrations of phenolics, flavonoids, tannins, alkaloids, and saponins, suggesting it as a more efficient solvent for obtaining a broad spectrum of bioactive compounds with pharmacological potential (Wakeel *et al.,* 2019).

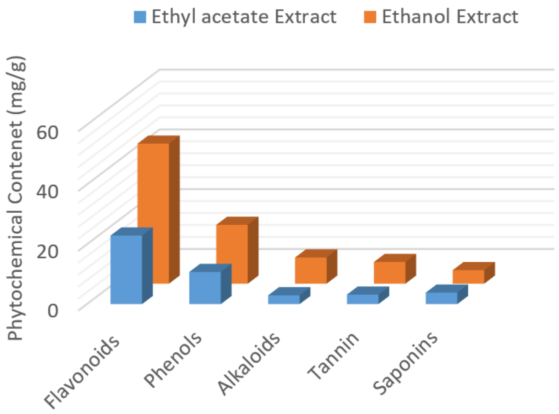


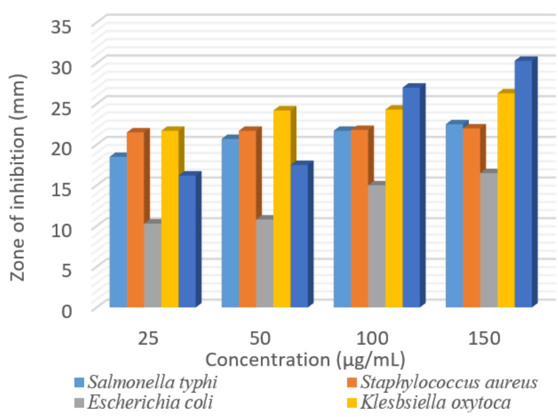
Figure 1. Quantitative phytochemical composition of ethyl acetate and ethanol extract of *Nauclea latifolia* leaves.
Source: Elaborated by the authors.

3.4. Antimicrobial screening

3.4.1. Analysis of antibacterial

The antibacterial activity of ethyl acetate and ethanol extracts was assessed at varying concentrations (25, 50, 100, and 150 µg/mL) against several bacterial strains, including *Salmonella typhi* ATCC 6539, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 *Klesbsiella oxytoca* ATCC 700324, *and Proteus vulgaris* ATCC 6380. The results were evaluated by measuring the zones of inhibition, as shown in **Fig. 2**. Both extracts demonstrated a dose-dependent effect against *Salmonella typhi* ATCC 6539, as evidenced by an increasing zone of inhibition with higher extract concentrations. In general, the inhibition zones of the ethanol extract were higher than those of the ethyl acetate extract. Regarding *Staphylococcus aureus* ATCC 25923, similar patterns have been observed, with concentration-dependent increases in the inhibitory zones in both extracts. Once more, ethanol extract performed better than ethyl acetate all the time. At increasing doses, both extracts showed a significant increase in inhibitory zones for *Escherichia coli* ATCC 25922. Compared to ethyl acetate, ethanol extracts consistently showed wider inhibition zones. For both extracts, *Klesbsiella oxytoca* ATCC 700324 displayed an increase in concentration-dependent inhibitory zones. Compared to ethyl acetate, ethanol extracts consistently exhibited wider inhibition zones. For both extracts, *Proteus vulgaris* ATCC 6380 showed a notable rise in inhibitory zones with concentration. Compared to ethyl acetate, ethanol extracts typically exhibited wider inhibition zones. Having been considered, the findings indicate that ethanol extracts work better than ethyl acetate extracts at preventing the growth of the microorganisms under investigation. These extracts may have antibacterial properties due to the concentration-dependent response, with ethanol extract often having greater potency (Biswas *et al.,* 2013). This outcome was in agreed with the investigation on the antibacterial activity of *Nauclea latifolia*, which found that two out of the four test organisms that were tested were resistant to the effects of the root and leaf polar solvent extracts (alcohol and aqueous) (Okwori *et al.,* 2008).

A



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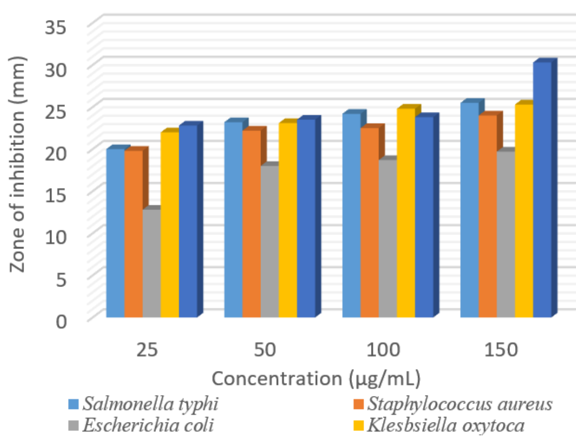

Figure 2. Zones of inhibition (mm) of antibacterial properties of **(a)** ethyl acetate extract and **(b)** ethanol extract, from Nauclea latifolia leaves against the concentration (µg/mL).
Source: Elaborated by the authors.

3.4.2. Analysis of antifungal

The data obtained for *Nauclea latifolia* leaf extract's antifungal activity using the mycelia inhibition method is shown in (**Fig. 3**). The antifungal results show that ethanol and ethyl acetate extracts have significant growth inhibition percentages against different fungal strains. Both solvent extracts exhibited antifungal activity. *Aspergillus niger* ATCC 1640, and *Aspergillus flavus* ATCC 9643 showed significant growth inhibition, with comparable percentages of 88% and 89%, respectively, highlighting the comparable potency of both extracts. *Penicillium venetum* ATCC 10431 and *Fusarium solani* ATCC 36031 both showed comparable percentages of 89% and 86%, respectively, demonstrating reliable efficacy, while *Fusarium solani* ATCC 36031 showed somewhat higher suppression (85%) with ethanol extract. The consistency of the outcomes across several fungal strains indicates the dependability of both extract in preventing fungal growth. These results confirm that the extracts have the potential to be potent antifungal agents, and the choice of solvent has no effect on how well they work. High inhibitory percentages are a constant indicator of the extracted antifungal activity. This emphasizes their potential as strong candidates for additional research in sectors concerned with managing fungal infections (Rashed *et al.,* 2021). These results are consistent with the study investigating the antifungal efficacy of *Nauclea diderrichii* methanol and dichloromethane extracts, exhibiting significant antifungal properties (Theophine *et al.,* 2017).

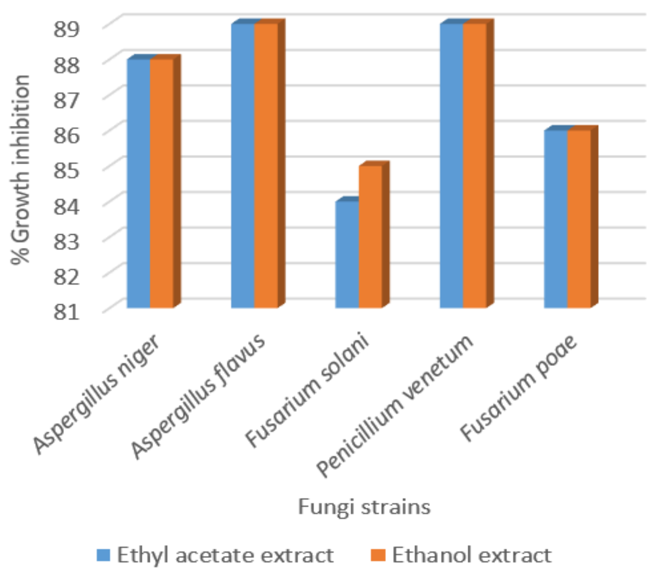

Figure 3. Antifungal activity study of leaves extracts of *Nauclea latifolia* on clinical isolate.
Source: Elaborated by the authors.

3.5. Antioxidant activity

3.5.1. DPPH free radical scavenging

The capacity of *Nauclea latifolia* leaf extracts to lower DPPH has been used to investigate their antioxidant potential. The mechanism by which antioxidant compounds interact with DPPH is based on the transfer of an electron or hydrogen atom to the DPPH radical, which results in the formation of 2,2-diphenyl-1-picrylhydrazine (Baliyan *et al.,* 2022; Gulcın and Alwasel, 2023). Reduced DPPH radicals occur in a discoloration that changes from purple to pale yellow, indicating scavenging action (Sadeer *et al.,* 2020). The antioxidant activity of ascorbic acid is shown in (**Table 3**) and the two solvent extracts were assessed at concentrations ranging from 0.5 to 3.5 mg/mL using the DPPH test.

Table 3. DPPH free radical scavenging activity of leaves extracts.

Extracts	0.5 (mg/mL)	2.0 (mg/mL)	3.5 (mg/mL)	IC_{50} (mg/mL)
Ethyl acetate	13.5±0.1	24.9±0.2	71±1	2.71
Ethanol	34.7±0.7	48.3±0.3	73±1	2.11
Ascorbic Acid	69.5±0.5	80.8±0.3	86.6±0.2	1.02

Source: Elaborated by the authors.

The DPPH radical scavenging properties of the extracts and ascorbic acid are shown in (**Table 3**). Ascorbic acid, used as the control, exhibited consistently high scavenging activity, ranging from percentage of 69.5 ± 0.5 to 86.6 ± 0.2. In comparison, the ethanol and ethyl acetate extracts displayed concentration-dependent radical scavenging activity, with ranges of percentage 34.7 ± 0.7 to 73 ± 1 and 13.5 ± 0.1 to 71 ± 1, respectively. Although the scavenging activity of the extracts was lower than that of ascorbic acid, the findings remain significant as they demonstrate the presence of notable antioxidant capacity in the extracts. The results highlight the potential of these extracts as natural antioxidants, especially when considered in the context of their complex phytochemical composition, which may provide complementary or synergistic effects in antioxidant-related therapeutic applications. The ethanol and ethyl acetate extracts' IC_{50} values were determined from the dose-response curves, and they were 2.11 and 2.71 mg/mL, respectively, while the IC_{50} of the standard antioxidant was 1.02 mg/mL. Low IC_{50} values are associated with high antioxidant activity (Brighente *et al.,* 2007). Ethanol extract exhibited the highest level of antioxidant activity, while ethyl acetate extract had the highest IC_{50} value. These results were entirely consistent with the research published, which found that the aqueous extracts of the fruits and leaves demonstrated the ability to scavenge free radicals (Ayeleso *et al., 2014*).

3.5.2. Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay measures an antioxidant's ability to convert Fe^{3+} into Fe^{2+} ions in the presence of TPTZ, forming a bright blue Fe^{2+}-TPTZ complex with maximum absorbance at 593 nm. Higher FRAP data indicate a more potent antioxidant capacity, suggesting that the antioxidant can protect cells from free radical damage and mitigate oxidative stress (Santos-Sánchez *et al.,* 2019). The antioxidant activity of ascorbic acid at concentrations ranging from 0.5 to 3.0 mg/mL and the two solvent extracts were evaluated using the FRAP test. **Table 4** presents the study's results.

Table 4. Ferric reducing antioxidant power of leaves extracts.

Extracts	0.5 (µg/mL)	2.0 (µg/mL)	3.5 (µg/mL)
Ethyl acetate	47±1	51.6±0.4	58.3±0.5
Ethanol	62.4±0.4	62.9±0.3	64±1
Ascorbic Acid	74±1	81.1±0.6	88.7±0.6

Source: Elaborated by the authors.

The antioxidant activity of the ethanol and ethyl acetate extracts increased with higher concentrations, ranging from 62.4 ± 0.4% to 64±1% and 47 ± 1% to 58.3 ± 0.5%, respectively. These values were lower than the standardized antioxidant activity of ascorbic acid, which ranged from 74±1% to 88.7 ± 0.6% (**Table 4**). Despite the difference in activity levels, the concentration-dependent increase observed in the extracts highlights their potential as natural antioxidants, offering significant activity that could be further optimized or used in combination with other antioxidant agents. FRAP evaluations are positively correlated with enhanced antioxidant capability. The extract's FRAP value was compared to the reference value. Notably, the ethanolic extract had the most potent antioxidant capacity. These results are consistent with the investigation conducted previously by Ayeleso *et al.* (2014) from which aqueous extracts from leaves and fruits showed the ability to reduce ferric ions, with leaves showing more activity than fruits (Ayeleso *et al.,* 2014).

3.5.3. Pancreatic α-amylase inhibitory assay

The percentage radical scavenging activity of pancreatic α-amylase inhibition was plotted against extract concentrations (**Table 5**) to estimate the IC_{50} values (**Table 5**). The IC_{50} value obtained for ethanol was 2.88 mg/mL, and 2.91 mg/mL for the ethyl acetate extract. The IC_{50} value of acarbose, the standard positive control, was 1.00 µg/mL. According to the pancreatic α-amylase inhibitory investigations, the ethanol and ethyl acetate extracts of *Nauclea latifolia* exhibit lower inhibitory potency when compared with the standard drug as a lower IC_{50} value indicates higher potency.

Table 5. Pancreatic α-amylase free radical scavenging activity of leaves extracts.

Extracts	0.5 (µg/mL)	2.0 (µg/mL)	3.5 (µg/mL)	IC_{50} (µg/mL)
Ethyl acetate	41.5±0.2	57.5±0.3	60.9±0.1	2.19
Ethanol	31.3±0.9	44.3±0.7	53±1	2.88
Acarbose	70±1	80.5±0.4	88.6±0.7	1.00

Source: Elaborated by the authors.

Based on these findings, the ethyl acetate extract exhibits more powerful inhibitory effects on α-amylase in comparison to the ethanol extract. Because of its lower IC_{50} value, the commercially available acarbose is also the most effective pancreatic α-amylase inhibitor. Understanding how these extracts may control the rate at which carbohydrates are metabolized is critical for managing conditions like diabetes. This result aligns with the previous study on the extract, where the polar solvent moderately inhibited the α-amylase (Akinwunmi *et al.,* 2019).

3.5.4. Bovine serum albumin denaturation assay

The BSA denaturation capacity of ethanol and ethyl acetate extracts was investigated, and the results were compared to ibuprofen, which was used as a standard. The findings, which are presented in (**Table 6**), demonstrate the notable efficiency of the *Nauclea latifolia* leaves ethyl acetate extract (IC_{50} = 2.52 mg/mL) over the ethanolic extract (IC_{50} = 4.62 mg/mL) in comparison to the standard Ibuprofen (IC_{50} = 1.76 mg/mL).

Table 6. Bovine serum albumin free radical scavenging activity of leaves.

Extracts	0.5 (µg/mL)	2.0 (µg/mL)	3.5 (µg/mL)	IC_{50} (mg/mL)
Ethyl acetate	33.6±0.3	45.4±0.1	61±1	2.52
Ethanol	15.5±0.2	26.7±0.5	37±1	4.62
Ibuprofen	43.4±0.2	56.6±0.3	80±1	1.76

Source: Elaborated by the authors.

The anti-inflammatory properties of the ethanol and ethyl acetate extracts were both dose-dependent (**Table 6**). However, the ethyl acetate extract was more effective because of its lower IC_{50}. This result is in complete agreement with previous studies, where moderate activity was noted (Iheagwam *et al.,* 2020). The current investigation highlights the potential of the ethyl acetate extract as a promising anti-inflammatory drug and provides important insights into the differences in efficacy between the ethanol and ethyl acetate extracts from *Nauclea latifolia*.

4. Conclusions

This study highlights the significant medicinal potential of *Nauclea latifolia* leaf extracts, which are rich in diverse secondary metabolites. Among the tested solvent extracts, the ethanolic extract demonstrated the highest composition of key bioactive compounds. The ethanolic extract exhibited remarkable antimicrobial activity against the selected pathogenic species, underscoring its potential as an effective antimicrobial agent. Additionally, it displayed strong antioxidant activity, evidenced by a low IC_{50} value of 2.11 mg/mL and strong reducing power. Furthermore, the ethyl acetate extract showed notable inhibition of pancreatic α-amylase (IC_{50} = 2.19 mg/mL) and bovine serum albumin denaturation (IC_{50} = 2.52 mg/mL), suggesting potential applications in managing conditions like diabetes and inflammation. This work provides valuable insights into the phytochemical profile and pharmacological properties of *Nauclea latifolia*, contributing to the understanding of its medicinal value. These findings underscore the importance of *Nauclea latifolia* in traditional medicine while providing a critical foundation for future research to isolate and characterize its bioactive compounds, advancing its potential in drug discovery and development for antimicrobial, anti-inflammatory, and antidiabetic therapies.

Authors' contribution

Conceptualization: Adewusi John Adepoju, Ibidotun Theophilus Olawoore; **Data curation**: Ibidotun Theophilus Olawoore, Taofeek Temitope Toromade; **Formal Analysis**: Ibidotun Theophilus Olawoore, Taofeek Temitope Toromade; **Funding acquisition**: Niyi Basil Omodara, Adewusi John Adepoju, I bidotun Theophilus Olawoore, Taofeek Temitope Toromade; **Investigation**: Adewusi John Adepoju, Ibidotun Theophilus Olawoore, Taofeek Temitope Toromade; **Methodology**: Adewusi John Adepoju, Ibidotun Theophilus Olawoore, Taofeek Temitope Toromade; **Project administration**: : Niyi Basil Omodara, Adewusi John Adepoju, Akintomiwa Olumide Esan; **Resources**: Adewusi John Adepoju, Ibidotun Theophilus Olawoore, Taofeek Temitope Toromade; **Software**: Not applicable; **Supervision**: Adewusi John Adepoju, Akintomiwa Olumide Esan; **Validation**: Adewusi John Adepoju, Akintomiwa Olumide Esan; **Visualization**: Akintomiwa Olumide Esan; **Writing – original draft**: Ibidotun Theophilus Olawoore; **Writing – review & editing**: Ibidotun Theophilus Olawoore, Adewusi John Adepoju.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability statement

The data will be available upon request.

Artificial Intelligence usage statement

The authors declare that no Artificial Intelligence (AI) tools or AI-assisted technologies were used in the preparation, writing, revision, grammar correction, language editing, data analysis, interpretation of results, or any other aspect of this manuscript. All content was conceived, written, reviewed, and approved solely by the authors. Furthermore, no AI tools were used during the preparation of revised versions of the manuscript submitted throughout the peer-review process.

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