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Nauclea latifolia Stem Bark Extracts: Potentially Effective Source of Antibacterial, Antioxidant, Antidiabetic and Anti-Inflammatory Compounds

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ABSTRACT: The stem bark of African peach (*Nauclea latifolia*) plant is used as traditional remedy for diabetes and inflammatory diseases, however, authenticated information on this use is scare. Therefore, the objective of this paper was to investigate the traditional folklore of the trado-medical remedy of the stem bark of African peach (*Nauclea latifolia*) plant for diabetes and inflammatory diseases using appropriate standard methods. Qualitative phytochemical screening confirmed the presence of saponins, alkaloids alkaloids, flavonoids, tannins, coumarin, steroids, terpenoids, cardiac glycosides, glycosides, quinones, anthocyanin, anthraquinone, and phenol in the extracts. The ethanol extract exhibited the highest quantities of phenolics (19.69±0.12 mgGAE/g), flavonoids (46.84±0.12 mgQE/g), alkaloids (8.76±0.10 mg/g), tannins (7.25±0.10 mgTAE/g), and saponins (4.53±0.13 mg/g). Both ethyl acetate and ethanol extracts showed potent antibacterial and significant antifungal potential against the chosen pathogenic species. The ethyl acetate extract showed superior antioxidant and anti-diabetic activities compared to the ethanol extract. However, the ethanol extract outperformed in terms of anti-inflammatory efficacy, although still below standard Ibuprofen. This study propounds that *N. latifolia* stem bark is an attainable diabetic medication due to its interactions with enzymes, antioxidants, and anti-inflammatory capabilities and its potential for isolating medicinal compounds.

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Medicinal plants have long been acknowledged as a significant source for the treatment of a variety of ailments, and they may even be regarded as the forerunners of contemporary medicine (Mintah *et al*., 2019; Salmeron-Manzano *et al.,* 2020; Theodoridis *et al.,* 2023). Even now, hundreds of plants are grown in various parts of the world to provide active ingredients for the pharmaceutical, cosmetic, food and dietary supplement industries (Salmeron-Manzano *et al.,* 2020). These days, many societies place a high value on medicinal plants. Most antidiabetics medications and around 80% of all medicines come from plants or plant-based bioactive substances (Vaou *et al.,* 2021; Asma *et al.,* 2022). The medicinal value of therapeutic plants and their phytoconstituents as natural sources is increasing due to their well-known potential to scavenge free radicals. Thus, plants are sources of natural antioxidant molecules with various pharmacological actions and few to no side effects that defend against many diseases (Omari *et al.,* 2019; Llaurado *et al.,* 2020). Phytochemicals are substances that naturally occur in plants and play a significant role in the defense mechanisms used by those plants to fend off diseases (Bansal and

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Priyadarsini, 2021). These phytochemicals are also beneficial to humans because they significantly impact biological processes in the body that are essential for healthy functioning, including immune function and a variety of biological processes (Pallie *et al.,* 2020). Phytochemicals such as polyphenols, alkaloids, flavonoids, saponins, terpenoids, steroids, tannins, quinones and cardiac glycosides are present in medicinal plants leaves, fruits, roots, flowers and seeds, as well as other parts of the plant (Sha'a *et al.,* 2019; Parameswari *et al.,* 2019).

These substances exhibit physiological properties, including antioxidant activity, antibacterial effect, modulation of detoxification enzymes, immune system stimulation, platelet aggregation reduction, hormone metabolism modulation, and antidiabetics and anti-inflammatory properties (Safari and Ahmady-Asbchin, 2019; Panda *et al.,* 2023; Talib *et al.,* 2022). *Nauclea latifolia* is a member of the Rubiaceae family and is a native of tropical Africa and Asia, it is popularly known in western, norther and eastern part of Nigeria as egbesi, tafashiya and ubulu inu respectively (Balogun *et al.,* 2013). It has a distinct flower and huge red fruit balls with protruding stamens. Although edible, the crimson fruit is unattractive.

N. latifolia is a multi-stemmed evergreen shrub or tree, it can reach a height of 200 m and it is commonly found in West and Central Africa's savannah forests and wet tropical rainforest zones (Balogun *et al.,* 2013). Since ancient times, many cultures have used the *N. latifolia* plant as a folk remedy for a variety of ailments.

These ailments include diarrhea, discomfort, dental cavities, an infected mouth, diabetes, tuberculosis, asthma, bronchitis, coughing and colds (Dike *et al.,* 2012; Oke *et al.,* 2021; Anarado *et al.,* 2022). Therefore, the objective of this paper was to investigate the traditional folklore of the trado-medical remedy of the stem bark of African peach (*Nauclea latifolia)* plant for diabetes and inflammatory diseases.

MATERIALS AND METHODS

Plant Collection: The plant sample of *N. latifolia* was collected in the hamlet of Ibapon along Odo-Oba in Ogbomosho, Oyo State, Nigeria, identified by Prof. A.T.J. Ogunkunle of the Pure and Applied Biology Department at Ladoke Akintola University of Technology Ogbomosho. The plant sample stem was cleaned with distilled water to remove any impurities, bark peeled, sliced into smaller pieces, air-dried for 40 days, pulverized and kept in an airtight container until further study.

Extraction: The pulverized plant material (1200 g) was first soaked with n-hexane for one week and the solvent was changed every seven days to ethyl acetate and ethanol until no extraction was observed. Then, each of the solvent extract was filtered and concentrated with a rotary evaporator, the weight of the extracts was obtained and recorded.

Qualitative phytochemical screening: The stem bark extracts of *N. latifolia* were screened for phytochemical constituents according to a standard method described by Shaikh and Patil, (2020). The phytochemicals tested for include saponins, alkaloids, flavonoids, tannins, coumarin, steroids, terpenoids, cardiac glycosides, glycosides, quinones, anthraquinone, anthocyanin and phenol.

Determination of total alkaloid contents (TAC): The method described by Van Tan (2018) was used to determine the presence of alkaloids. 200 mL of 10 % acetic acid in ethanol was added to 5 g each of the sample, which had been weighed into a 250 mL beaker. The mixture was then covered and let to stand for 4 hours. This was filtered, and the extract was then concentrated in a water bath to a quarter of its initial volume. Drop by drop, concentrated ammonium hydroxide was added to the extract until the precipitation was completed. The entire solution was allowed to settle, and the precipitate was then collected, cleaned with diluted ammonium hydroxide, and filtered. The alkaloid, which was dried and weighed, is the final substance.

Determination of total saponin contents (TSC): The Obadoni and Ochuko (2002) methodology was adopted. A conical flask was filled with 100 cm^3 of 20 % aqueous ethanol and 20 g of each ground sample after the samples had been processed. The samples were stirred and boiled for 4 hours at roughly 55 ^{0}C over a hot water bath. After filtering the mixture, 200 mL of 20 % ethanol was used to extract the residue. Approximately 90 °C water bath was used to decrease the combined extracts to 40 mL. 20 mL of diethyl ether was added, followed by the concentrate, and forcefully agitated in a 250 mL separatory funnel. While discarding the ether layer, the aqueous layer was recovered. The purification procedure was repeated. n-butanol 60 mL was added. Two separate washes of 10 mL of 5% aqueous sodium chloride were performed on the combined n-butanol extracts. In a water bath, the remaining 35 solution was warmed. Following evaporation, the samples were baked to a consistent weight, and the percentage of saponin was determined.

Determination of total phenolic content (TPC): The total quantity of phenolics in plant extracts was

determined using spectrophotometric analysis. According to Siddiqui *et al.* (2017), the total phenol content was determined using the Folin-Ciocalteu test method. In a volumetric flask (25 mL), the reaction mixture of 1 mL of extract and 9 ml of distilled water was taken. The mixture was treated with one milliliter of Folin-Ciocalteu phenol reagent and thoroughly shaken. 10 mL of a 7% sodium carbonate (Na_2CO_3) solution was added to the mixture after 5 minutes. A 25 mL volume was created. In the same way as previously described, a series of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 g/mL) were created. A UV/Visible spectrophotometer was used to measure the absorbance of the test and standard solutions at 550 nm in comparison to the reagent blank after 90 minutes of incubation at room temperature. The amount of total phenol was calculated as mg of GAE/g of extract.

Determination of total tannin Content (TTC): The Folin-Ciocalteu method was used to determine the tannins, according to Rao, (2016). A volumetric flask (10 mL) containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu Phenol reagent, 1 mL of 35 % Na2CO3 solution, and 10 mL of distilled water was filled with the sample extract in an amount of around 0.1 mL. After thoroughly shaking, the mixture was left at room temperature for 30 minutes. In the same way as previously stated, a set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 g/mL) were created. A UV/Visible spectrophotometer measured absorbance for test and standard solutions against the blank at 725 nm. The amount of tannin in each gram of extract was measured in mg of GAE.

Determination total flavonoid content (TFC): According to Shraim *et al.* (2021), the aluminium chloride colourimetric assay was used to determine the total flavonoid concentration. In a 10 mL volumetric flask, the reaction mixture of 1 mL of extract and 4 mL of distilled water was added. After five minutes, 0.3 ml of 10 % aluminium chloride was added to the flask and 0.30 ml of treated 5% sodium nitrite. After 5 minutes, 2 mL of 1M sodium hydroxide was treated and diluted to 10 mL with distilled water. The same procedure as previously described was used to generate a set of reference standard solutions of quercetin at concentrations of 20, 40, 60, 80 and 100 g/mL. Using a UV/Visible spectrophotometer, the absorbance of the test and standard solutions was measured at 510 nm compared to the reagent blank.

Antibacterial screening: According to the National Committee for Clinical Laboratory Standards (Harathi *et al.,* 2017), the ethyl acetate and ethanol extracts of Nauclea latifolia stem bark were tested for their ability to inhibit the growth of Gram-positive and Gram-negative bacteria, including *Escherichia coli, salmonella typhi, Klebsiella oxytoca, staphylococcus aureus* and *proteus vulgaius*, using the agar well diffusion method. All pathogens were isolated from clinical specimens provided by the BOWEN University Teaching Hospital in Ogbomosho, Oyo State, Nigeria's microbiology department. By subculturing the pure isolates in nutrient agar and incubating them for 24 hours at 37 °C for bacteria, the test organisms were individually generated. 25, 50, 100 and 150 mg/mL were the concentrations that were employed. The sensitivity test's criterion was the absence of development on or around the plate. Before observing and measuring the zone of inhibition in millimetres, the plates were incubated at 37 ^{0}C for 24 hours.

Antifungal screening: The mycelia growth inhibition test was used to study ethanolic and ethyl acetate extracts' antifungal properties (Synytsya *et al.,* 2017). Potato dextrose agar (PDA) was prepared with extracts added at a 25 g/ml concentration. The PDA plates in the central portion of this study were inoculated with a 6 mm agar plug containing 48-hour-old cultures of *Aspergillus niger, Aspergillus fumigatus, Fusarium solani, Penicillium novetatum,* and *Aspergillus flavus*. Empty nanoparticles were present in the control plates. Every plate went through a 72-hour incubation period at 28 °C. To compute the percentage growth inhibitions, the radial fungal growths on all the plates were measured, and the following formula was used:

$$\%GI = \frac{Dcontrol - Dtest}{Dcontrol} x\ 100\% \quad (1)$$

Where GI = percent growth inhibitions; D is the diameter of fungal growth on the PDA plates.

Determination of DPPH Free Radical Scavenging Activity: According to Oyedemi *et al.* (2010), different concentrations of the stem bark extracts of *N. latifolia* in a methanolic solution of DPPH were used to measure the free radical scavenging and antioxidant activities of the stem bark extracts of *N. latifolia* against the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

A spectrophotometer was used to detect the absorbance at 517 nm after 30 min of incubation at room temperature in the dark, using vitamin C as a reference substance. Using the following equation, the percentage inhibition for each concentration of extracts and the standard was determined.

$$\% \text{ Inhibition} = \left(\frac{Ab - As}{Ab}\right) \times 100 \quad (2)$$

Where A_s is the absorbance of various concentrations of the stem bark extracts of *N. latifolia* and A_b is the absorbance of the blank solution. The extracts and the standard's IC50 values were computed after the dose-response curve was plotted.

Ferric reducing antioxidant power assay (FRAP): Jemli *et al.* (2016) guidelines for the FRAP assay were followed with a few adjustments. The stock solutions contained a 300nm acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL of acetic acid), pH 3.6, and a ten mM solution of 2,4,6-tripyridyl-s-triazine. Acetate buffer, 2.5 mL of TPTZ solution, and 25 mL of $FeCl_3.6H_2O$ solution were combined to create the new working solution. The plant extract sample (50 µL, 1 mg/mL) and FRAP solution were combined, and the mixture was allowed to react for 5 minutes in the dark. The colourful substance (ferrous tripyridyltriazine complex) was then measured at 593 nm. Trolox equivalents in milligrams (mg TE/g) were used to evaluate the results.

Anti-inflammatory Assay: Bovine serum albumin assay (BSA): A modified version of the BSA assay described by Williams *et al.* (2008) was used to determine the anti-inflammatory properties of the crude and fractionated plant extracts. BSA solution (0.4 percent, w/v) was made in Tris Buffered Saline by dissolving one tablet in 15 mL of deionized water to make 0.05 milligrams of Tris and 0.15 milligrams of sodium chloride, which results in a pH of 7.6 at 25 °C. Glacial acetic acid was used to bring the pH level down to 6.4. Each plant extract was produced as stock solutions in methanol at a concentration of 50 µg/mL, or 0.005%, w/v. Test tubes containing 1 mL of 0.4%, w/v BSA buffer solution were filled with the corresponding aliquots of 5.0 µL, 10 µL and 20 µL, which represented concentrations of 0.25 µg/mL, 0.50 µg/mL and 1.00 µg/mL of the stock solutions. Aspirin was a positive control, and methanol was a negative control.

The solutions were then heated for 10 minutes in a water bath at 72 °C and cooled for 20 minutes in a lab setting. Using an air blank and a Hach Spectrophotometer, the turbidity of the solutions (level of protein precipitation) was determined at 660 nm. The studies were carried out twice and mean absorbance values were noted. The following calculation was used to calculate the percentage inhibition of precipitation (protein denaturation) in comparison to the negative control:

$$\% ADA = \left(\frac{\text{ABC} - \text{ABS}}{\text{ABC}}\right) \text{x } 100 \quad (3)$$

Where ADA = Anti-Denaturation Activity; ABC = absorbance Control; ABS = absorbance of sample

Anti-Denaturation Activity = % Inhibition of Protein Denaturation = % Anti-inflammatory Activity

Anti-diabetics Assay: α-Amylase Inhibitory Assay: Minor adjustments were made to the standard methods for conducting the study (Hansawasdi *et al.,* 2000). 2 mg of starch azure was suspended in 0.2 mL of 0.5 M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ (substrate solution). The tubes containing the substrate solution underwent a 5-minute preincubation at 37°C. After boiling for 5 minutes. An extract sample was dissolved in DMSO to produce different extract concentrations. The substrate solution tube was then topped off with 0.2 mL of a particular plant extract concentration. In addition, 0.1 mL of swine pancreatic amylase in Tris-HCl buffer (2 units/mL) was added to the tube holding the plant extract and substrate solution. The reaction was run at 37°C for 10 minutes. The process was stopped by adding 0.5 mL of 50% acetic acid to each tube. The reaction mixture was centrifuged at 3000 rpm for 5 minutes at 4 °C. The absorbance of the resulting supernatant at 595 nm was measured using a spectrophotometer. Ascorbic acid, a well-known inhibitor of α-amylase, was given as a standard prescription. The experiments were carried out three times. The inhibitory activity of -amylase was calculated using the following formula:

The α-amylase inhibitory activity =

$$\frac{[(Ac+)-(Ac-)]-[(As-Ab)]}{[(Ac+)-(Ac-)]} \times 100 \quad (4)$$

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.

RESULTS AND DISCUSSION

Percentage yield of the N. latifolia extracts: Table 1 shows the *N. latifolia* stem bark extraction yield. The yield in percentages for n-hexane, ethyl acetate, and ethanol are 0.48 %, 0.58 % and 1.31 %, respectively.

The relationship between a solvent's polarity and unique ability to penetrate plant cell walls and extract phytochemicals from them is suggested by the fact that the percentage yield increased as the solvent's polarity increased. This outcome also made

it evident that ethanol, a more polar solvent, can extract more phytochemicals.

Table 1: Percentage yield of *N. latifolia* stem bark extract with different solvents

Solvent extracts	Solvent yield (g)	Percentage yield
EHE	8.09	0.48
EAE	9.87	0.58
EEE	22.25	1.31

Key: EHE- n-Hexane extracts, EAE- Ethyl acetate extract, EEE- Ethanol extract

Qualitative phytochemical screening of the extracts: The results of the phytochemical screening of *N. latifolia* stem bark extract is given in Table 2. All the secondary metabolites (saponins, alkaloids, flavonoids, tannins, coumarin, steroids, terpenoids, cardiac glycosides, glycosides, quinones, anthraquinone, anthocyanin and phenol) were absent from the plant's n-hexane crude extracts. glycosides and anthraquinone were absent from the crude extract of ethyl acetate, but saponins, alkaloids, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, glycosides, quinones, anthocyanin and phenol were all present in the crude extract of ethanol. This outcome is consistent with earlier research by Iheagwam *et al.* (2020) which found alkaloids, saponins, tannins, flavonoids, anthocyanin, quinones, cardiac glycosides and glycosides in the leaves extract of *N. latifolia*. These plants secondary metabolites may be what gives medicinal plants their biological activity (Hussein and El-Anssary, 2019).

Table 2: Phytochemical composition of stem bark extracts of *N. latifolia*

Phytoconstituents	EHE	EAE	EEE
Saponins	–	+	+
Alkaloids	–	+	+
Flavonoids	–	+	+
Tannins	–	+	+
Coumarin	–	+	+
Steroids	–	+	+
Terpenoids	–	+	+
Cardiac Glycosides	–	+	+
Glycosides	–	–	+
Quinones	–	+	+
Anthraquinone	–	–	+
Anthocyanin	–	+	–
Phenol	–	+	+

Key: EHE- n-Hexane extracts, EAE- Ethyl acetate extract, EEE- Ethanol extract, + represent (present) and - represent (absent).

Quantitative Analysis: In this study, the total phenolic content (TPC), total flavonoid content (FTC), total tannin content (TTC), total alkaloid content (TAC) and total saponin content (TSC) of the crude extracts are showed in Table 3. The data in the table showed that phenol, tannin, flavonoid, alkaloid, and saponin quantities were higher in the ethanol extract of *N. latifolia* than in the ethyl acetate extract. Flavonoids had the highest quantity of secondary metabolites (46.84 mgQE/g) compared to ethyl acetate extract (22.93 mgQE/g). There have been numerous reports of flavonoids' biological effects. These include cytotoxic antitumor, therapy for neurological disorders, anti-inflammatory, antibacterial, antiviral and anti-allergic properties (Maleki *et al.,* 2019; Fei *et al.,* 2014; Kawai *et al.,* 2007); and vasodilatory impact (Sak, 2014; Maher, 2019; Duarte *et al.,* 1993). Furthermore, flavonoids reduce platelet aggregation, lipid peroxidation, capillary permeability, and fragility. They also reduce the activity of the enzymes cyclo-oxygenase and lipoxygenase. They perform those functions as divalent cation chelators, antioxidants and free radical scavengers (Mucha *et al.,* 2021). They are also known to block several enzymes, including hydrolases, hyaluronidase, alkaline phosphatase, arylsulphatase, cAMP phosphodiesterase, lipase, and -glucosidase (Barber *et al.,* 2021).

The second-highest quantity of secondary metabolite of *N. latifolia* stem bark is phenols (10.77 mgGAE/g for the ethyl acetate extract and 19.69 mgGAE/g for the ethanol extract). The phenolic chemical family, which includes polyphenols, is one of the most significant and prevalent groups of plant metabolites. The secondary metabolites polyphenols are the most widely distributed because they contain thousands of chemicals. Numerous phenolics have been shown to exhibit elevated levels of antioxidant activity (Wu and Wang, 2001). The biological effects of phenolic compounds, which have been studied because they have the potential to be antioxidants and free radical scavengers, are of particular interest. Additionally, phenolic chemicals play a role in pathogen or UV radiation resistance. The ethanol extract (7.25 mgTAE/g) of the stem bark of *N. latifolia* had more tannins overall than the ethyl acetate extract which was also found to contain (3.19 mgTAE/g) of tannins. Numerous fungi, yeasts, bacteria, and viruses are also prevented from growing by tannins. The astringent activity of these tannins, which promotes quicker wound and mucous membrane healing, is also a factor (Ashok and Upadhyaya, 2012). Tannins may have antibacterial and antiviral effects (Vilhelmova-Ilieva *et al.,* 2020; Farha *et al.,* 2020).

Alkaloids protect against chronic diseases (Aryal *et al.,* 2022). By preventing DNA topoisomerase from functioning, alkaloids, a varied category of secondary metabolites, have been demonstrated to exhibit antibacterial activity (Barbieri *et al.,* 2017). Ethanol extract and ethyl acetate extract of *N. latofolia* stem bark was determined and was found to had the quantity of alkaloids (8.76 mg/g) and (2.94 mg/g) respectively. The lowest secondary metabolite in the stem bark of

N. latifolia is saponin, which is present in extracts of ethyl acetate (3.84 mg/g) and ethanol (4.53 mg/g). Saponins have strong foaming and emulsifying properties as well as a range of pharmacological effects, including antibacterial, virucidal, anti-inflammatory, anti-leishmaniosis, anti-cancer, etc. They also serve as phytoprotectors against a number of diseases. (Sharma *et al.,* 2017). According to Timilsena *et al.* (2023), saponins exhibit a wide range of biological activity based on their distinctive chemical properties, such as their potent affinity for the phospholipids in cell membranes, their capacity to form insoluble complexes with sterols and proteins, their capacity to have lytic activity, etc. The higher levels of the studied secondary metabolites in ethanol extract may be explained by ethanol's ease with which it may penetrate cell membranes and draw out intracellular components from plant materials.

Table 3: Quantitative results for phenols, tannin, flavonoids, alkaloid and saponin of *N. latifolia* stem bark

Phytochemicals	EAE	EEE
Phenol (mgGAE/g)	10.77±0.13	19.69±0.12
Flavonoid (mgQE/g)	22.93±0.10	46.84±0.12
Tannin (mgTAE/g)	3.19±0.10	7.25±0.10
Alkaloid (mg/g)	2.94±0.12	8.76±0.10
Saponin (mg/g)	3.84±0.12	4.53±0.13

Key: EAE- Ethyl acetate extract, EEE- Ethanol extract

Antibacterial Evaluation: Table 4 shows the various zones of inhibition of *Nauclea latifolia* stem bark extract in ethyl acetate and ethanol at various concentrations on various microorganisms. Ethyl acetate extract has an inhibition zone between 9.0 mm and 25.0 mm, whereas ethanol has an inhibition zone between 9.0 mm and 21.5 mm. The *Nauclea latifolia* stem bark extracts in ethyl acetate and ethanol are both harmful to bacteria strains (*Bacellus subtilis, Bacillus megaterium, Escherichia coli, Klebsiella oxytoca, and Proteus mirabilis*), with ethyl acetate being more sensitive than ethanol extract. *Proteus mirabilis* had the smallest zone of inhibition (14.5 mm) and *Klebsilla oxytoca* had the largest zone of inhibition (25.5 mm) when exposed to ethyl acetate extract at 8 mg/mL. With a zone of inhibition of 20.5 mm, Bacillus subtilis showed the highest sensitivity to ethanol extract compared to other microbes. *Escherichia coli* displayed the lowest zone of inhibition at 13.5 mm at 8 mg/mL. The findings clearly showed that the ethyl acetate extract was more effective at killing the tested organisms. This observation regarding the antibacterial activity of plant extracts may be related to the age of the plant used, the freshness of the plant materials, physical factors (temperature, light, and water), the timing of the plant materials harvesting, and the drying technique used before the extraction process (Altemimi *et al.,* 2017). The antibacterial activity of the stem bark extract against these bacteria (*Bacillus subtilis, Bacillus megaterium, Escherichia coli, Klebsiella oxytoca and Proteus vulgaris*) may result from the presence of all these secondary metabolites with therapeutic value because the pharmacological activity of many medicinal plants has been reported to be directly correlated with the types of secondary metabolites they contain (Teoh, 2016).

Table 4: Antibacterial activity study of stem bark extracts of N. latifolia on clinical isolate.

Zone of Inhibition in diameter (mm)						
	Concentration (mg/mL)					
Organisms	**Ethyl acetate Extract**			**Ethanol Extract**		
	4	6	8	4	6	8
Bacillus subtilis	9.0	12.5	21.5	14.0	16.5	20.5
Bacillus megaterium	9.5	11.5	15.5	9.5	11.0	14.5
Escherichia coli	12.5	15.0	18.5	9.0	13.0	13.5
Klebsiella oxytoca	9.0	18.5	25.5	10.0	11.5	17.5
Proteus vulgaris	10.5	11.5	14.5	10.5	14.5	17.5

Antifungal studies: The data obtained for *N. latifolia* stem bark extracts antifungal activity using the mycelia inhibition method is shown in Table 5. The extracts demonstrated good antifungal potential with inhibition activities ranging from 77 to 90 %. The table also shows that the ethanolic and ethyl acetate extracts had only moderate effects on the tested strains; the solvent's polarity may also explain this observation. While *Aspergillus fumigates* (86 %) were least affected by both the ethanolic and the ethyl acetate extract (86 %), the highest activity was observed against the growth of *Aspergillus parasitics* (90 %) for both extracts. The presence of numerous bioactive components in the extract, such as alkaloids, can be ascribed to the plant extracts antifungal activity (Gizaw *et al.,* 2022).

Table 5: Antifungal activity study of stem bark extracts of *N. latifolia* on clinical isolate.

	Growth Inhibition (%)	
Organisms	Ethyl acetate extract	Ethanol extract
Aspergillus Fumigattus	86	86
Aspergillus flavus	88	89
Fusarium solani	78	77
Aspergillus parasitics	90	90

DPPH Free radical scavenging: The antioxidant activity of stem bark extracts of *N. latifolia* has been studied by its ability to reduce DPPH. Interaction of antioxidant compounds with DPPH is based on the

transfer of hydrogen atom or electron to DPPH radical and converts it to 2, 2- diphenyl-1- picrylhydrazine (Baliyan *et al.,* 2022; Gulcin and Alwasel, 2023). The result of reduction DPPH radicals causes discoloration from purple color to yellow pale color which demonstrates the scavenging activity (Sadeer *et al.,* 2020). With concentrations ranging from 500 to 3500 µg/mL, the antioxidant activity of the two solvent extracts and ascorbic acid was tested against the DPPH assay, with the results shown in Table 3. The ethyl acetate and ethanol extracts, as shown in Table 6, both showed comparable and significant concentration-dependent free radical scavenging activity, with values ranging from 54.05±1.80 to 77.56±0.34 and 58.20±1.06 to 71.18±0.55, respectively, in comparison to the standard ascorbic acid's value of 69.47±0.54 to 86.60±0.23. For the ethyl acetate and ethanol extracts, the IC_{50} values for the DPPH assay were 13.63 and 15.98 µg/mL, respectively. The IC_{50} of the common antioxidant was 10.21 µg/mL. High antioxidant activity is correlated with low IC_{50} values (Brighente *et al.,* 2007). In contrast to ethyl acetate extract, which had the greatest IC_{50} value, ethanol extract showed the strongest antioxidant activity.

Table 6: DPPH free radical scavenging activity of stem bark extracts of N. latifolia.

Extracts	500 µg/mL	2000 µg/mL	3500 µg/mL	IC_{50}
EAE	54.05±1.80	68.92 ± 1.23	77.56 ± 0.34	13.63
EEE	58.20 ± 1.06	64.77 ± 1.55	71.18 ± 0.55	15.98
Ascorbic Acid	69.47 ± 0.54	80.80 ± 0.28	86.60 ± 0.23	10.21

Key: *EAE- Ethyl acetate extract, EEE- Ethanol extract, Ascorbic Acid- Standard drug*

Ferric reducing antioxidant power assay (FRAP): The ferric reducing antioxidant power (FRAP) assay is based on antioxidants capacity to reduce Fe^{3+} into Fe^{2+} ions in the presence of TPTZ, resulting in the formation of a bright blue Fe^{2+}-TPTZ complex with the maximum absorbance at 593 nm. Higher FRAP data have a more potent antioxidant capacity as a result. These indicate that it can mitigate oxidative stress and defend cells from damage brought on by free radicals (Santos-Sanchez *et al.,* 2019). The antioxidant activity of the two solvent extracts and ascorbic acid was evaluated using the FRAP assay at doses ranging from 500 to 3500 g/ml, with the results presented in Table 7.

Table 7: Ferric Reducing Antioxidant Power of stem bark extracts of *N. latifolia*

Extracts	500 µg/ml	2000 µg/ml	3500 µg/ml
EAE	62.29±1.16	67.74±2.43	75.01±2.94
EEE	57.32±2.32	65.45±2.22	75.07±1.62
Ascorbic Acid	74.29±2.06	81.07±1.29	88.69±1.20

Key: EAE- Ethyl acetate extract, EEE- Ethanol extract, Ascorbic Acid- Standard drug

According to Table 7, the antioxidant activity of the ethanol and ethyl acetate extracts increased as their concentration increased, ranging from 62.29±1.16 to 75.01±2.94, respectively, in comparison to the value of standard ascorbic acid, which ranged from 74.29±2.06 to 88.69±1.20. A higher FRAP value is associated with vigorous antioxidant activity. They were comparing the extracts FRAP value to the reference value. The highest antioxidant capacity is seen in ethyl acetate extract.

α-Amylase Inhibitory Assay: The IC_{50} values were determined Table 8, by plotting the percentage of α-amylase inhibition as a function of extract concentrations. The IC_{50} for the crude ethyl acetate extract was 20.91 µg/mL, while the IC_{50} for ethanol was 30.59 µg/mL. Acarbose was used as the standard positive control, and its IC_{50} value was 9.96 µg/ml. The α-amylase inhibitory studies showed that the ethanol and ethyl acetate extracts of *N. latifolia* had significant inhibitory potentials. According to these results, the ethyl acetate extract demonstrates more potent inhibitory effects on α-amylase than the ethanol extract. Acarbose, the commercial standard drug, is also the most effective alpha-amylase inhibitor, as seen by its lower IC_{50} value. This knowledge is essential for comprehending how these extracts might regulate how quickly carbohydrates are digested, which may be necessary for managing illnesses such as diabetes. This finding corresponds precisely with the earlier research on the plants ripe fruit methanolic extract by Akinwunmi *et al.* (2019), where the polar solvent moderately inhibited the α-amylase.

Bovine Serum Albumin Denaturation Assay: The BSA denaturation activity of the ethanol and ethyl acetate extracts of *N. latifolia* was studied; the results were contrasted with those of ibuprofen, which was used as the reference. According to the results of the study Table 9, the ethanol extract of *N. latifolia* stem bark, with an IC_{50} value of 16.38 µg/mL, is significantly more effective than both standard Ibuprofen (IC_{50} of 17.59 µg/mL) and an ethyl acetate extract in preventing heat-induced albumin denaturation. Both the ethanol and ethyl acetate extracts have dose-dependent anti-inflammatory effects, with the ethanol extract being more effective due to its lower IC_{50}. This outcome perfectly aligns with the earlier research on the plants leaf extract by Iheagwam *et al.* (2020), where the extracts show a moderate activity.

Table 8: α-amylase free radical scavenging activity of stem bark extracts of *N. latifolia*

Extracts	500 µg/ml	2000 µg/ml	3500 µg/ml	IC_{50}
EAE	35.73 ± 2.82	46.07 ± 0.27	59.40 ± 1.40	20.91
EEE	27.53 ± 0.71	42.40 ± 2.43	50.67±2.72	30.59
Acarbose	70.44 ± 1.19	80.50 ± 0.79	88.61 ± 1.30	9.96

Key: EAE- Ethyl acetate extract, EEE- Ethanol extract, Ascorbic Acid- Standard drug

Table 9: Bovine serum albumin free radical scavenging activity of stem bark extracts of N. latifolia.

Extracts	500 µg/ml	2000 µg/ml	3500 µg/ml	IC_{50}
EAE	53.18 ± 1.24	58.92 ± 1.65	62.94 ± 0.53	19.61
EEE	60.12 ± 0.88	61.42±1.12	70.16 ± 0.72	16.38
Ibuprofen	43.38 ± 0.71	56.59 ± 0.94	79.73 ± 2.95	17.59

Key: EAE- Ethyl acetate extract, EEE- Ethanol extract, Ibuprofen- Standard drug

Conclusion: Phytochemical analysis of *N. latifolia* stem bark indicated the presence of alkaloids, steroids, flavonoids, saponins, and phenols, implying medicinal applications. The extracts exhibited antibacterial and antifungal activity against tested microorganisms, highlighting potential use in microbial-triggered diseases. The study also confirmed potent antioxidant, anti-inflammatory, and anti-diabetic effects, aligning with the plant's total phenolic content. These results substantiate the traditional medicinal use of the plant and validate reported antioxidant effects in the literature.

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