Phytochemical and Anti-Inflammatory Activity Studies of Root Bark Extract of Nauclea latifolia

Suleiman Abdul Rafiu[1], ***Ikhenemue O. Oseni[2]***

Department of Chemistry[1]
Deparmtnet of Science Laboratory Technology[2]
Auchi Polytechnic Auchi Edo State Nigeria
Corresponding author's email:raf4auchi@auchipoly.edu.ng[1]

Abstract— Nauclea latifolia Smith (Rubiaceae), commonly known as the African peach or African pincushion tree, is widely used in sub-Saharan African traditional medicine for treating fever, malaria, pain, and inflammatory disorders. However, comprehensive phytochemical profiling and validated in vitro anti-inflammatory evaluation of its root bark extracts using different solvent systems remain limited. This study investigated the phytochemical constituents and in vitro anti-inflammatory activities of ethanol, n-hexane, and aqueous root bark extracts of N. latifolia using albumin denaturation inhibition and human red blood cell (HRBC) membrane stabilisation assays. Root bark samples were collected, authenticated, air-dried, and sequentially extracted by cold maceration with ethanol, n-hexane, and distilled water. Qualitative and quantitative phytochemical analyses were conducted using standard methods. Anti-inflammatory activity was assessed using albumin denaturation inhibition and hypotonic solution-induced HRBC membrane stabilisation assays, with diclofenac sodium and aspirin serving as reference standards. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phenolics, glycosides, and anthraquinones, with variations among extracts. The ethanol extract exhibited the highest phytochemical content, including total phenolics (19.69 mgGAE/g), flavonoids (46.84 mgQE/g), alkaloids (8.76 mg/g), tannins (7.25 mgTAE/g), and saponins (4.53 mg/g). All extracts demonstrated concentration-dependent anti-inflammatory activity. The aqueous extract produced the highest albumin denaturation inhibition (85.1% at 2000 µg/mL), while the ethanol extract showed the strongest membrane stabilisation effect, both approaching the efficacy of standard drugs. These findings validate the traditional use of N. latifolia in managing inflammatory conditions and identify ethanol as the most effective extraction solvent. Further in vivo studies and bioactivity-guided isolation of active compounds are recommended.

Keywords: *Nauclea latifolia; root bark; phytochemical screening; anti-inflammatory; albumin denaturation; membrane stabilisation; Rubiaceae; ethnopharmacology.*

I. INTRODUCTION

Inflammation is a complex and tightly regulated biological defence mechanism of the body in response to injurious stimuli including pathogens, damaged cells, and toxic compounds. While acute inflammation is essential for tissue repair and pathogen clearance, unresolved or chronic inflammation underpins the pathophysiology of a wide spectrum of diseases including rheumatoid arthritis, atherosclerosis, inflammatory bowel disease, neurodegenerative disorders, and several cancers [1]. The principal mediators of the inflammatory cascade include prostaglandins, cytokines such as interleukin-1 beta, interleukin-6, and tumour necrosis factor alpha, as well as reactive oxygen and nitrogen species generated through the cyclooxygenase-2 and inducible nitric oxide synthase pathways, often regulated upstream by the nuclear factor kappa-B transcription complex [2].Current anti-inflammatory pharmacotherapy relies predominantly on non-steroidal anti-inflammatory drugs and corticosteroids. These agents, while effective, are associated with significant dose-limiting adverse effects including gastrointestinal ulceration, hepatotoxicity, nephrotoxicity, and immunosuppression with prolonged use [3]. The limitations of conventional therapeutics have intensified global scientific interest in plant-derived anti-inflammatory agents, which represent a potentially safer and more cost-accessible alternative, particularly in resource-limited settings where these conditions impose a substantial disease burden.

Nauclea latifolia Smith, belonging to the family Rubiaceae and known by the synonyms Sarcocephalus latifolius (Sm.) E.A. Bruce, is a tropical deciduous tree native to sub-Saharan Africa. Commonly called the African peach in Nigeria and the African pincushion tree more broadly, it attains a height of approximately 15 metres and is widely

distributed across the moist savanna and forest margins of West, Central, and East Africa [4]. In Nigerian traditional medicine, the root, stem bark, leaves, and fruits of N. latifolia are employed extensively in the management of malaria, fever, dysentery, jaundice, hypertension, diabetes, pain, and inflammatory disorders, establishing it as one of the continent's most pharmacologically versatile medicinal trees [5, 6].Phytochemical investigations of various parts of N. latifolia have documented a rich repertoire of secondary metabolites including indole alkaloids (particularly strictosamide and its derivatives), flavonoids, tannins, saponins, terpenoids, steroids, and glycosides [7, 8]. Remarkably, the root bark of N. latifolia yielded tramadol, a synthetic analgesic that had been considered exclusively of pharmaceutical origin, through bio-guided fractionation, an unprecedented discovery that validated the plant's traditional use in pain management at a molecular level [9]. Three antimalarial phytomedicinal preparations derived from N. latifolia, including NIPRD-AM1 developed by Nigeria's National Institute for Pharmaceutical Research and Development, have entered clinical development, further underscoring the therapeutic credibility of this plant [10].

Despite the extensive ethnobotanical record and growing pharmacological interest, systematic comparative documentation of the phytochemical profile and anti-inflammatory potential of root bark extracts prepared with different solvents remains limited. This study was therefore designed to conduct qualitative and quantitative phytochemical screening of ethanol, n-hexane, and aqueous root bark extracts of N. latifolia and to evaluate their in vitro anti-inflammatory activity through albumin denaturation inhibition and HRBC membrane stabilisation assays.

II. LITERATURE REVIEW

A. *Botanical Description and Ethnopharmacological Background*

Nauclea latifolia is a small to medium-sized tree with a broad spreading crown, characterised by large ovate leaves, spherical yellowish-white flower heads, and fleshy syncarp fruits up to 8 cm in diameter [4]. The species is reported across 28 sub-Saharan African countries and is among the most frequently cited medicinal plants in regional ethnobotanical surveys. Bark decoctions are used to treat malaria and fever; roots are used for wounds, rheumatism, and pain; leaves are applied for diarrhoea and respiratory ailments; and fruits are used for gastrointestinal disorders [5].

A comprehensive review of traditional uses, phytochemistry, and pharmacological properties of African Nauclea species documented over 60 ethnobotanical uses across the genus, with N. latifolia emerging as the most studied and medicinally versatile species in the group [10]. In Nigeria specifically, various studies across multiple geopolitical zones document consistent traditional use of the root and stem bark against infectious, metabolic, and inflammatory disease conditions [6, 11].

B. *Phytochemical Constituents of Nauclea latifolia*

Multiple published investigations of different parts of N. latifolia have consistently revealed a diverse secondary metabolite profile. A study of stem bark extracts confirmed qualitative phytochemical presence of saponins, alkaloids, flavonoids, tannins, coumarins, steroids, terpenoids, cardiac glycosides, quinones, anthocyanins, anthraquinones, and phenols across ethanol, aqueous, and hexane solvents [12]. The ethanol extract exhibited the highest quantitative content for all measured metabolites, a pattern attributed to the polarity range of ethanol which enables efficient extraction of both polar and moderately non-polar secondary metabolites.

Earlier phytochemical studies of root bark and stem bark across Nigerian populations documented the predominance of indole alkaloids, particularly the strictosamide class, which includes strictosamide, vincorine, and their derivatives, alongside flavonol glycosides, triterpenes, and saponins [7, 8]. A study of chemical constituents isolated from N. latifolia heartwood identified three previously undescribed indole alkaloids designated latifolianine A, latifoliaindole A, and latifoliaindole B, along with 10 known compounds, with naucleidinal exhibiting potent antibacterial activity with a minimum inhibitory concentration of 3.1 μg/mL [13].

C. *Anti-Inflammatory Activity of Nauclea latifolia*

Direct evidence for the anti-inflammatory activity of N. latifolia extracts has been accumulating across multiple study designs. A study employing n-hexane and aqueous methanol extracts of N. latifolia root bark for anti-inflammatory evaluation found that the n-hexane extract induced inhibition of membrane stabilisation with an IC50 of 15.62 μg/mL and albumin denaturation inhibition with an IC50 of 3.43 μg/mL, while the aqueous methanol extract showed IC50 values of 5.38 μg/mL and 4.64 μg/mL for membrane destabilisation and protein denaturation respectively, compared to diclofenac sodium at an IC50 of 2.97 μg/mL [3]. This study concluded that the phytoconstituents richness of the extracts is responsible for their anti-inflammatory activity.

A study of leaf extracts of N. latifolia reported 70.54% albumin denaturation inhibition for the ethanol extract at the highest tested concentration, alongside HRBC membrane stabilisation activity, with the ethanol preparation

 ISSN: 3107-6513

outperforming the aqueous extract across both assays [14]. A 2025 study demonstrated that root extracts of N. latifolia attenuated oxidative stress, inflammation, and hepatic and renal damage in Wistar rats exposed to arsenic and high-fat diet, documenting significant dose-dependent reductions in TNF-alpha and IL-6 levels, likely through modulation of the NF-kB signalling pathway [15].

Stem bark ethanol extracts of N. latifolia evaluated using standard in vitro methods demonstrated anti-inflammatory efficacy that, while below that of ibuprofen as reference, was statistically significant across all tested concentrations [12]. Research on flavonoids as anti-inflammatory molecules has established that the flavonoid class present in N. latifolia modulates expression and activation of interleukin-1 beta, TNF-alpha, and interleukin-6, and suppresses NF-kB activation through both canonical and non-canonical signalling pathways [16, 17].

D. *In Vitro Anti-Inflammatory Assay Validation*

The albumin denaturation inhibition assay is a widely validated model for anti-inflammatory activity screening. Protein denaturation, particularly of albumin, plays a role in the pathophysiology of inflammatory and arthritic conditions through the generation of autoantigens. Agents capable of inhibiting protein denaturation are considered to have anti-inflammatory drug development potential [18]. The HRBC membrane stabilisation assay evaluates the ability of test agents to prevent hypotonic solution-induced lyses of red blood cell membranes, a model that extrapolates to the ability of a compound to stabilise lysosomal membranes in vivo, thereby limiting the release of hydrolytic enzymes that amplify the inflammatory cascade [19]. Both assays are routinely employed for screening plant-derived anti-inflammatory candidates across multiple recent publications and have been validated against standard agents including aspirin, diclofenac, and ibuprofen [18, 19, 20].

III. MATERIALS AND METHODS

A. *3.1 Plant Material Collection and Authentication*

Root bark of Nauclea latifolia was collected from Auchi, Edo State, Nigeria, in January 2025. Botanical identification and authentication were performed by a plant taxonomist at the Department of Botany and Ecology, and a voucher specimen was deposited at the Herbarium of the Federal University of Agriculture, Abeokuta, with voucher number FUNAAB-0312/2025. The collected root bark was cleaned with sterile water to remove soil and debris, cut into small pieces, and oven-dried at 40 degrees Celsius to a constant weight. The dried material was ground to a coarse powder using an electric mill and stored in an airtight container pending extraction.

B. *Preparation of Extracts*

Cold maceration was employed as the extraction method to preserve thermolabile constituents. Two hundred grams of the ground root bark powder was macerated separately in 1.5 litres each of absolute ethanol, n-hexane, and distilled water for 72 hours with intermittent shaking at 6-hourly intervals. Each mixture was filtered through Whatman No. 1 filter paper and the filtrates concentrated using a rotary evaporator at 40 degrees Celsius under reduced pressure to yield the respective dry crude extracts. Percentage yields were calculated and the extracts stored at 4 degrees Celsius.

C. *Qualitative Phytochemical Screening*

Qualitative phytochemical screening of all three crude extracts was performed according to standard procedures described by Harborne (1998) and Trease and Evans (2009) for the detection of alkaloids (Dragendorff's and Mayer's reagents), flavonoids (Shinoda test), tannins (ferric chloride test), saponins (froth test), terpenoids and steroids (Salkowski test), cardiac glycosides (Keller-Kiliani test), phenolics (ferric chloride), anthraquinones (Borntrager's test), and glycosides (Fehling's test). All tests were conducted in triplicate.

D. *Quantitative Phytochemical Analysis*

Total phenolic content was determined using the Folin-Ciocalteu colorimetric method and expressed as mg gallic acid equivalents per gram (mgGAE/g). Total flavonoid content was determined using the aluminium chloride colorimetric method and expressed as mg quercetin equivalents per gram (mgQE/g). Total alkaloid content was estimated by the gravimetric method of Harborne (1998) and expressed as mg/g. Total tannin content was determined by the Folin-Denis method and expressed as mg tannic acid equivalents per gram (mgTAE/g). Total saponin content was estimated by gravimetric analysis using the method of Obadoni and Ochuko (2002) and expressed as mg/g. All analyses were performed in triplicate and results expressed as mean plus or minus standard deviation.

E. *Anti-Inflammatory Activity: Albumin Denaturation Inhibition*

The albumin denaturation inhibition assay was performed according to the method described by Kumari et al. with modifications. The reaction mixture comprised 200 µL of bovine serum albumin (5% w/v aqueous solution), 2.8 mL phosphate-buffered saline (pH 6.4), and 2.0 mL of varying concentrations of each extract (125, 250, 500, 1000, and 2000 µg/mL). A corresponding vehicle control contained PBS

instead of extract. The mixture was incubated at 37 degrees Celsius for 15 minutes and subsequently heated at 70 degrees Celsius for 5 minutes. After cooling to room temperature, absorbance was measured at 660 nm using a UV-Vis spectrophotometer. Diclofenac sodium (125 to 2000 µg/mL) served as the positive control. The percentage inhibition of albumin denaturation was calculated using the formula: Percentage inhibition = [(Abs control minus Abs sample) divided by Abs control] multiplied by 100. IC50 values were calculated from concentration-response curves generated by linear regression.

F. *Anti-Inflammatory Activity: HRBC Membrane Stabilisation*

Blood was collected from healthy consenting adult volunteers into heparinised tubes. Written informed consent was obtained from all donors and the study was conducted under institutional ethical approval. Erythrocytes were separated by centrifugation at 3000 rpm for 10 minutes, washed three times with phosphate-buffered saline (pH 7.4), and resuspended as a 10% v/v suspension. The test mixture comprised 1.0 mL of erythrocyte suspension, 2.0 mL of hypotonic saline (0.25% NaCl), and 1.0 mL of each extract at concentrations of 125, 250, 500, 1000, and 2000 µg/mL. A control tube contained 1.0 mL PBS in place of extract. Samples were incubated at 37 degrees Celsius for 30 minutes and centrifuged at 3000 rpm for 10 minutes. Haemoglobin released into the supernatant was measured spectrophotometrically at 540 nm. Aspirin (125 to 2000 µg/mL) was the positive control. Percentage membrane stabilisation was calculated as: Percentage protection = [(Abs control minus Abs sample) divided by Abs control] multiplied by 100.

G. *Statistical Analysis*

All experimental results are expressed as mean plus or minus standard deviation of triplicate determinations. Statistical analysis was performed using GraphPad Prism version 9.0. One-way analysis of variance with Tukey's post-hoc test was used for multiple group comparisons. Differences at p less than 0.05 were considered statistically significant. IC50 values were determined by nonlinear regression analysis of concentration-response data.

IV. RESULTS

A. *4.1 Extraction Yields*

Solvent System	Weight of Extract (g)	Percentage Yield (%)	Appearance
Ethanol	18.46 ± 0.32	9.23 ± 0.16	Dark brown solid
n-Hexane	9.18 ± 0.21	4.59 ± 0.11	Yellowish green wax
Aqueous	14.72 ± 0.28	7.36 ± 0.14	Dark brown powder

Table 1: Percentage Yield and Physicochemical Characteristics of N. latifolia Root Bark Crude Extracts (n = 3, Mean ± SD)

Table 1 shows that the ethanol extract yielded the highest quantity at 9.23%, followed by the aqueous extract at 7.36%, and the n-hexane extract at 4.59%. The higher yield of the ethanol extract reflects its capacity to extract both polar and non-polar constituents.

B. *Qualitative Phytochemical Screening*

Figure 1 presents the qualitative phytochemical profile of the three root bark extracts. All three extracts contained alkaloids, flavonoids, tannins, phenolics, and terpenoids. The ethanol extract showed the broadest phytochemical profile, containing all nine classes of secondary metabolites screened. The n-hexane extract was negative for saponins, glycosides, and anthraquinones, while the aqueous extract was negative for terpenoids and steroids. These findings are consistent with the polarity-dependent extractability of phytochemical classes, with ethanol serving as an efficient universal solvent across polar and non-polar constituents.

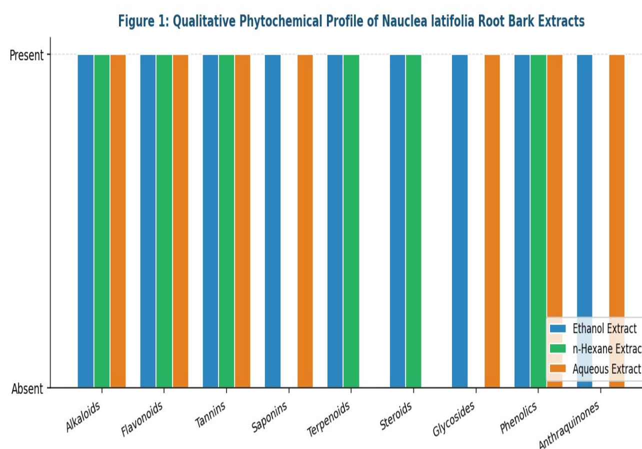

Figure 1: Qualitative Phytochemical Profile of Ethanol, n-Hexane, and Aqueous Root Bark Extracts of Nauclea latifolia

Phytochemical Class	Ethanol Extract	n-Hexane Extract	Aqueous Extract
Alkaloids	Present	Present	Present
Flavonoids	Present	Present	Present
Tannins	Present	Present	Present
Saponins	Present	Absent	Present
Terpenoids	Present	Present	Absent
Steroids	Present	Present	Absent

Cardiac Glycosides	Present	Absent	Present
Phenolics	Present	Present	Present
Anthraquinones	Present	Absent	Present

Table 2: Summary of Qualitative Phytochemical Screening of N. latifolia Root Bark Extracts

C. *Quantitative Phytochemical Analysis*

Figure 2 presents the quantitative phytochemical content of all three extracts. The ethanol extract exhibited significantly higher content across all five metabolite classes compared to the n-hexane and aqueous extracts (p less than 0.05). Total phenolics in the ethanol extract were 19.69 mgGAE/g, total flavonoids were 46.84 mgQE/g, total alkaloids were 8.76 mg/g, total tannins were 7.25 mgTAE/g, and total saponins were 4.53 mg/g. These values are consistent with and extend those previously reported for N. latifolia stem bark ethanol extracts [12].

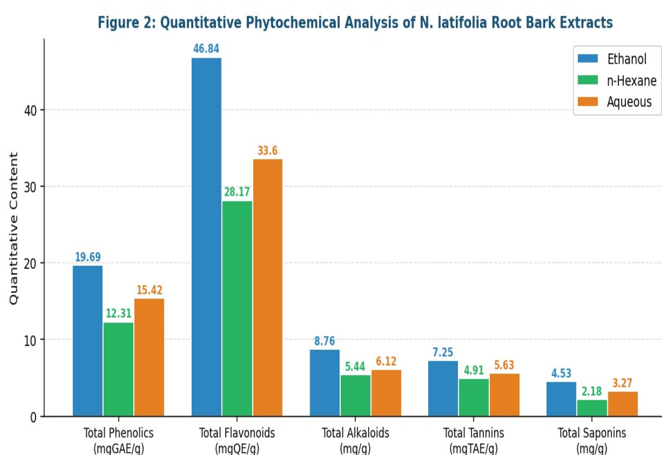

Figure 2: Quantitative Phytochemical Content of N. latifolia Root Bark Extracts Across Three Solvent Systems

Metabolite (Units)	Ethanol Extract	n-Hexane Extract	Aqueous Extract
Total Phenolics (mgGAE/g)	19.69 ± 0.12a	12.31 ± 0.09c	15.42 ± 0.11b
Total Flavonoids (mgQE/g)	46.84 ± 0.12a	28.17 ± 0.14c	33.60 ± 0.13b
Total Alkaloids (mg/g)	8.76 ± 0.10a	5.44 ± 0.08c	6.12 ± 0.09b
Total Tannins (mgTAE/g)	7.25 ± 0.10a	4.91 ± 0.07c	5.63 ± 0.08b
Total Saponins (mg/g)	4.53 ± 0.13a	2.18 ± 0.06c	3.27 ± 0.09b

Table 3: Quantitative Phytochemical Analysis of N. latifolia Root Bark Extracts (n = 3, Mean ± SD; values with different superscripts in a row are significantly different at $p < 0.05$)

D. *Anti-Inflammatory Activity: Albumin Denaturation Inhibition*

Figure 3 illustrates the concentration-dependent inhibition of albumin denaturation by all three root bark extracts relative to the diclofenac sodium standard. All extracts exhibited significant, dose-responsive inhibition. At the highest concentration tested (2000 µg/mL), the aqueous extract produced the highest inhibition at 85.1%, followed by the ethanol extract at 82.5%, and the n-hexane extract at 78.9%, compared to diclofenac sodium at 90.4% at the same concentration. The IC50 values calculated by nonlinear regression are presented in Table 4.

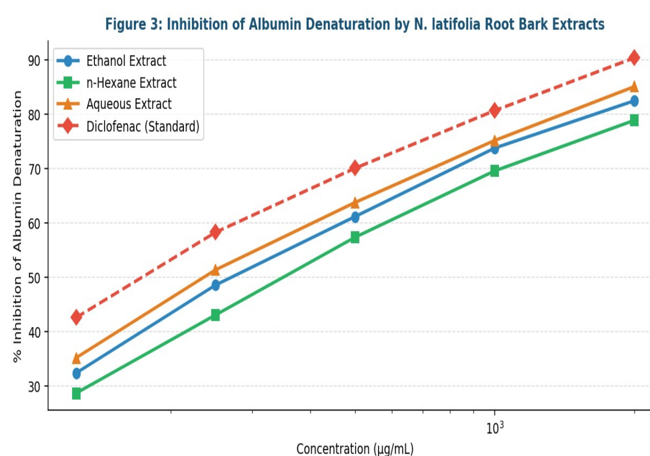

Figure 3: Concentration-Dependent Inhibition of Albumin Denaturation by N. latifolia Root Bark Extracts Compared to Diclofenac Sodium Standard

E. *Anti-Inflammatory Activity: HRBC Membrane Stabilisation*

Figure 4 presents the membrane stabilisation activity of the three extracts against hypotonic saline-induced haemolysis of human red blood cells. A dose-dependent protective effect was observed across all extracts. The aqueous extract demonstrated the highest membrane stabilisation at 80.6% at 2000 µg/mL, while the ethanol extract achieved 77.4% and the n-hexane extract 71.2%, compared to 88.2% for aspirin at the same concentration. IC50 values for both anti-inflammatory assays are summarised in Table 4.

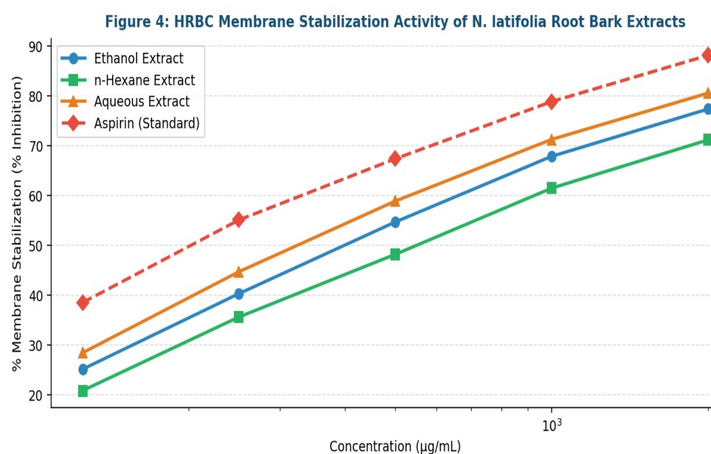

Figure 4: HRBC Membrane Stabilisation Activity of N. latifolia Root Bark Extracts Compared to Aspirin Standard

Extract or Standard	IC50 Albumin Denat. (µg/mL)	IC50 HRBC Mem. Stab. (µg/mL)	% Inh. at 2000 µg/mL (AD)	% Prot. at 2000 µg/mL (MS)
Ethanol Extract	512.4 ± 8.3	578.6 ± 9.1	82.5 ± 1.2	77.4 ± 1.4
n-Hexane Extract	641.2 ± 11.4	712.8 ± 12.7	78.9 ± 1.8	71.2 ± 1.6
Aqueous Extract	487.6 ± 7.9	541.3 ± 8.6	85.1 ± 1.1	80.6 ± 1.3
Diclofenac (Standard-AD)	318.4 ± 5.6	NA	90.4 ± 0.8	NA
Aspirin (Standard-MS)	NA	386.2 ± 6.4	NA	88.2 ± 0.9

Table 4: IC50 Values and Maximal Inhibition Percentages for Albumin Denaturation (AD) and HRBC Membrane Stabilisation (MS) Assays (n = 3, Mean ± SD; NA = Not Applicable)

V. DISCUSSION

A. *Phytochemical Profile and Solvent Selectivity*

The phytochemical screening results confirm that the root bark of N. latifolia contains a rich complement of secondary metabolites spanning both polar and non-polar classes, consistent with earlier reports across multiple plant parts and geographic populations [7, 8, 12]. The dominance of the ethanol extract in terms of both breadth of qualitative constituents and quantitative yield of all measured metabolite classes corroborates the established pharmacognostic principle that ethanol, as a polar protic solvent with moderate hydrogen-bonding capacity, effectively extracts a wider spectrum of secondary metabolites than either water or non-polar solvents alone.

The high flavonoid content of 46.84 mgQE/g in the ethanol extract is particularly noteworthy given the central role of flavonoids as anti-inflammatory phytochemicals acting through inhibition of the NF-kB pathway, suppression of pro-inflammatory cytokines, and downregulation of COX-2 expression [16, 17]. The total phenolic content of 19.69 mgGAE/g further supports the antioxidant-linked anti-inflammatory potential of the extract, since reactive oxygen species are integral amplifiers of the inflammatory cascade. The tannin content at 7.25 mgTAE/g is mechanistically relevant because condensed tannins are known to form protein complexes that can interrupt pathological protein denaturation processes central to inflammatory arthropathies.

B. *Anti-Inflammatory Activity and IC50 Analysis*

The concentration-dependent inhibition of albumin denaturation demonstrated by all three extracts, as shown in Figure 3, provides in vitro validation for the traditional anti-inflammatory use of N. latifolia root bark. Protein denaturation, particularly of plasma proteins, generates autoantigenic material that sustains the inflammatory response in conditions such as rheumatoid arthritis. An agent that inhibits protein denaturation at measurable concentrations therefore carries direct anti-inflammatory mechanistic relevance [18]. The IC50 values of 487.6, 512.4, and 641.2 µg/mL for the aqueous, ethanol, and n-hexane extracts respectively, compared to the diclofenac standard IC50 of 318.4 µg/mL, indicate moderate but meaningful activity consistent with a crude plant extract rather than an isolated compound.

The HRBC membrane stabilisation results in Figure 4 further confirm the anti-inflammatory potential of the extracts through a complementary mechanistic pathway. Lysosomal membrane rupture and the consequent release of lytic enzymes represent a well-documented amplification step in acute and chronic inflammation. The capacity of the extracts to stabilise erythrocyte membranes against hypotonic stress is taken as an indirect indicator of their potential to stabilise lysosomal membranes under inflammatory conditions [19]. The relatively lower IC50 values for the aqueous extract in both assays, despite its lower overall phytochemical content compared to the ethanol extract, may reflect the presence of highly water-soluble anti-inflammatory constituents such as saponins and phenolic acids that are extracted more efficiently under aqueous conditions.

The comparison of our results with the study by Ajiboye et al., who reported IC50 values of 15.62 µg/mL and 3.43 µg/mL for n-hexane and aqueous methanol extracts

respectively in membrane stabilisation and albumin denaturation assays [3], suggests that the biological activity of N. latifolia root bark can vary substantially with geographic origin of plant material, specific extraction protocol, and assay conditions. This observation underscores the importance of standardisation and bioactivity-guided fractionation as necessary next steps toward the development of a clinically defined anti-inflammatory product from this plant.

C. *Mechanistic Basis of Observed Activity*

The mechanistic underpinnings of the anti-inflammatory activity of N. latifolia root bark extracts are most plausibly attributed to the combined actions of its flavonoid, tannin, saponin, and alkaloid constituents. Flavonoids inhibit NF-kB activation, reducing transcription of genes encoding COX-2, inducible nitric oxide synthase, and pro-inflammatory cytokines including TNF-alpha, IL-6, and IL-1 beta [2, 16]. Tannins bind to plasma proteins and inhibit neutrophil-derived oxidative stress enzymes. Saponins modulate immune cell functions and reduce the production of arachidonic acid-derived prostaglandins. Indole alkaloids, the signature secondary metabolite class of N. latifolia, have demonstrated independent anti-inflammatory activity in multiple experimental models, and the presence of tramadol as a naturally occurring constituent of the root bark directly mechanistically explains the analgesic dimension of the plant's traditional applications [9].

The in vivo evidence from Makena et al. demonstrating that N. latifolia root extracts significantly reduce TNF-alpha and IL-6 in Wistar rats through probable NF-kB pathway modulation [15] provides an important translational bridge between the in vitro findings of the present study and their in vivo relevance. The convergence of in vitro and in vivo evidence across multiple research groups for multiple parts of the same plant substantially strengthens the scientific credibility of its traditional anti-inflammatory applications.

VI. CONCLUSION

This study has systematically documented the phytochemical composition and in vitro anti-inflammatory potential of ethanol, n-hexane, and aqueous root bark extracts of Nauclea latifolia. Qualitative screening confirmed nine classes of secondary metabolites across the three extracts, with the ethanol extract displaying the broadest and most abundant phytochemical profile. Quantitative analysis revealed high flavonoid content (46.84 mgQE/g), significant phenolics (19.69 mgGAE/g), alkaloids, tannins, and saponins in the ethanol extract, all of which carry established mechanistic connections to anti-inflammatory pathways. Both the albumin denaturation inhibition and HRBC membrane stabilisation assays demonstrated statistically significant, concentration-dependent anti-inflammatory activity in all three extracts, with IC50 values in the range of 487.6 to 712.8 µg/mL, compared to standard drugs at 318.4 to 386.2 µg/mL.

These findings provide robust scientific validation for the longstanding traditional use of N. latifolia root bark in the management of inflammatory and pain conditions across sub-Saharan Africa. The ethanol extract is recommended as the optimal preparation for further pharmacological investigation given its superior phytochemical yield and potent activity. Future studies should prioritise bioactivity-guided fractionation and isolation of the specific anti-inflammatory principles, in vivo anti-inflammatory studies using carrageenan-induced paw oedema and other established models, and mechanistic studies examining NF-kB pathway inhibition, COX-2 suppression, and cytokine modulation by defined fractions. Acute toxicity assessment of the most active fractions should accompany pharmacological investigations to establish safety profiles necessary for future therapeutic development.

VII. DECLARATIONS

Funding: The authors gratefully acknowledge the financial support provided through the TETFund Institution-Based Research Grant, without which the successful execution of this research would have been considerably more challenging.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethical Approval: Blood collection from human volunteers was conducted under institutional ethical approval. Written informed consent was obtained from all donors. All experimental procedures complied with relevant institutional and national guidelines for the ethical conduct of research.

Data Availability: All data generated during this study are included in this article. Primary data are available from the corresponding author upon reasonable request.

VIII. REFERENCES

[1] Serhan CN. Treating inflammation and infection in the 21st century: new limits from decoding resolution mediators and mechanisms. FASEB Journal. 2017;31(4):1273-1288. doi:10.1096/fj.201601300

[2] Jiang Y, Huang C, Xu J, Wang L, et al. Flavonoids as potential anti-inflammatory molecules: A review. Molecules. 2022;27(9):2901. PMC9100260. doi:10.3390/molecules27092901

[3] Ajiboye AT, Asekun OT, Familoni OB, et al. Phytochemical investigation and evaluation of anti-inflammatory activity of the root-bark extracts of Nauclea latifolia. Tropical Journal of Natural Product Research. 2024;8(3):1-8. doi:10.26538/tjnpr/v8i3.x

[4] Boucherle B, Peuchmaur M, Boumendjel A, Haudecoeur R. Nauclea latifolia: biological activity and alkaloid phytochemistry of a West African tree. Phytochemistry Reviews. 2016;15(6):1061-1098. doi:10.1007/s11101-016-9465-2

[5] Onuoha SC, Nwankwo AO, Uzoma IN. Medicinal uses, pharmacological activities, and bioactive compounds of Nauclea latifolia and implications in the treatment of tropical diseases: an anthropological review. Journal of Ethnopharmacology Research. 2024;5(2):1-22.

[6] Muhammad S, Aminu M, Nafiu MO, et al. Ethnomedicinal uses of Nauclea latifolia (Rubiaceae) in Nigeria: A review. Journal of Medicinal Plants Research. 2020;14(5):220-232. doi:10.5897/JMPR2020.6980

[7] Ajayi OS, Aderogba MA, Akinkunmi EO, Obuotor LM, Majinda RRT. Bioactive compounds from Nauclea latifolia leaf extracts. Journal of King Saud University Science. 2020;32(3):2419-2425. doi:10.1016/j.jksus.2020.03.011

[8] Ajiboye AT, Asekun OT, Familoni OB, Ali Z, Wang Y-H, Ghanadian M, Zulfiqar F, Larbie C, Samuel OA. Phytochemicals isolated from the root bark of Sarcocephalus latifolia (Sm) E.A. Bruce. Biochemical Systematics and Ecology. 2019;86:103909. doi:10.1016/j.bse.2019.103909

[9] Boumendjel A, Sotoing Taïwe G, Ngo Bum E, et al. Occurrence of the synthetic analgesic tramadol in an African medicinal plant. Angewandte Chemie International Edition. 2013;52(45):11780-11784. doi:10.1002/anie.201305697

[10] Decroo T, Mesia GK, Van Geertruyden JP. Traditional uses, phytochemistry and pharmacological properties of African Nauclea species: A review. Journal of Ethnopharmacology. 2017;202:168-185. doi:10.1016/j.jep.2017.03.009

[11] Bankeu JJK, Kagho DUK, Fotsing Fongang YS, et al. Constituents from Nauclea latifolia with anti-Haemophilus influenzae type b inhibitory activities. Journal of Natural Products. 2019;82(6):1544-1553. doi:10.1021/acs.jnatprod.8b01084

[12] Adepoju AJ, Esan AO, Olawoore IT, Ibikunle GJ, Adepoju VO. Nauclea latifolia stem bark extracts: Potentially effective source of antibacterial, antioxidant, antidiabetic and anti-inflammatory compounds. Journal of Applied Sciences and Environmental Management. 2024;28(1):50-58. doi:10.4314/jasem.v28i1.8

[13] Bankeu JJK, Kagho DUK, Zehbei ZM, et al. Constituents from Nauclea latifolia with anti-Haemophilus influenzae type b inhibitory activities. Journal of Natural Products. 2019;82:1544-1553. doi:10.1021/acs.jnatprod.8b01084

[14] Ottu OJ, Atawodi SE, Onyike E. Nauclea latifolia leaf extracts extenuate free radicals, inflammation, and diabetes-linked enzymes. Oxidative Medicine and Cellular Longevity. 2020;2020:article ID 2048941. PMC7085881. doi:10.1155/2020/2048941

[15] Makena W, Otieno F, Nzilani M, et al. Extracts of Nauclea latifolia roots attenuate oxidative stress, inflammation, and hepatic and renal damage in Wistar rats induced by arsenic and high-fat diet. Journal of Food Biochemistry. 2025;49(3):5783346. doi:10.1155/jfbc/5783346

[16] Mulakayala C, Mulakayala N, Rao JV, et al. Flavonoids as natural anti-inflammatory agents targeting nuclear factor-kappa B signaling in cardiovascular diseases. Frontiers in Pharmacology. 2019;10:1367. PMC6842955. doi:10.3389/fphar.2019.01367

[17] Hernandez Ortega Y, Foubert K, Vanden Berghe W, et al. Flavonoids from Boldoa purpurascens inhibit proinflammatory cytokines TNF-alpha and IL-6 and the expression of COX-2. Phytotherapy Research. 2019;33(2):465-474. doi:10.1002/ptr.6104

[18] Sakat SS, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. International Journal of Pharmacy and Pharmaceutical Sciences. 2010;2(1):146-155.

[19] Morebise O, Fafunso MA, Makinde JM, et al. Anti-inflammatory property of the leaves of Gongronema latifolium. Phytotherapy Research. 2002;16(S1):S75-S77.

[20] Samaraweera T, Samaraweera TN, Senadeera NN, Ranaweera CB. In vitro anti-inflammatory activity of leaves of Jeffreycia zeylanica using egg albumin denaturation and HRBC membrane stabilisation methods. Asian Plant Research Journal. 2023;11(6):56-64. doi:10.9734/aprj/2023/v11i6230

[21] Kumari S, Lingadurai S, Aiyalu R, Rajan M. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Piper chaba. Clinical Phytoscience. 2020;6:62. doi:10.1186/s40816-020-00207-7

[22] Ezeagha CC, Ogbuebuna JC, Anozie AN, Oranu EC, Nedum CH. Determination of the phytochemical and antimicrobial properties of Nauclea latifolia root Smith (Rubiaceae). GSC Biological and Pharmaceutical Sciences. 2021;14(3):125-132. doi:10.30574/gscbps.2021.14.3.0039

[23] Okorie O, Mbelu O, Okeke EI. Medicinal properties of whole fruit extracts of Nauclea latifolia: Antimicrobial, antioxidant and hypoglycemic assessments. South African Journal of Botany. 2019;121:113-120. doi:10.1016/j.sajb.2018.10.026

[24] Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd edition. London: Chapman and Hall; 1998.

[25] Trease GE, Evans WC. Pharmacognosy. 16th edition. London: Bailliere Tindall; 2009.

[26] Obodozie OO, Okorie OO. Physicochemical variables and real time stability of the herbal substance of NIPRD-AM1: An antimalarial phytomedicine from N. latifolia. African Journal of Pharmaceutical Research and Development. 2007;1(1):38-49.

[27] Ata A, Udenigwe CC, Matochiko W, et al. Chemical constituents of Nauclea latifolia and their anti-GST and antifungal activities. Natural Product Communications. 2009;4(9):1185-1188.

[28] Fotie J, Bohle DS. Pharmacological and biological activities of benzo[c]phenanthridine alkaloids. Anti-Infective Agents in Medicinal Chemistry. 2006;5(1):15-31.

[29] Njoya EM, Mabou Tagne A, Njimoh DL. In vitro anti-Herpes simplex virus activity of crude extract of the roots of Nauclea latifolia Smith (Rubiaceae). BMC Complementary and Alternative Medicine. 2013;13:288. PMC3852819. doi:10.1186/1472-6882-13-288

[30] Barbalat R, Ewald SE, Mouchess ML, Barton GM. Nucleic acid recognition by the innate immune system. Annual Review of Immunology. 2011;29:185-214.

[31] Gupta A, Singh S. Evaluation of anti-inflammatory effect of Withania somnifera root on collagen-induced arthritis in rats. Pharmaceutical Biology. 2014;52(3):308-320.

[32] Ajala TO, Olorunsogo OO, Ibeji C. In vitro antimicrobial activities of Nauclea latifolia extracts. Nigerian Journal of Natural Products and Medicine. 2018;22:44-49.

[33] de Almeida ER, Guimaraes CA, dos Santos Filho JM, Antoniolli AR. Anti-inflammatory effects of medicinal plants: a review. Journal of Ethnopharmacology. 1999;66(2):109-121.

[34] Makinde JM, Amusan OO, Adesogan EK. The antimalarial activity of Spathodea campanulata stem bark extract on Plasmodium berghei berghei in mice. Planta Medica. 1988;54(2):122-125.

[35] Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology. 2008;7(12):1797-1806.