Polarity-guided fractionation of *Lophira lanceolata* stem bark: Differential membrane-stabilizing, protein-denaturation inhibitory and antimicrobial activities

O. L. Chukwuemerie*[1], H. C. Nnadi[1], A. O. Ezigbo[2], M. C. Olise[1], N. E. Nnadozie[1], M. Okezie[3] and E. S. Iloh[4]

[1]Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[2]Department of Clinical Pharmacy, David Federal University, Uburu, Ebonyi State, Nigeria.
[3]Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[4]Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

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**Corresponding Author: Ogechukwu Lucy Chukwuemerie; ol.chukwuemerie@unizik.edu; +2347030529221*

Abstract
Lophira lanceolata is used in African traditional medicine, but the contribution of solvent polarity to the biological activity of its stem bark remains insufficiently defined. The methanol stem-bark extract was partitioned into n-hexane, ethyl acetate, n-butanol and aqueous fractions and evaluated by qualitative phytochemical tests, protein-denaturation and erythrocyte membrane-stabilization assays, agar-well diffusion, minimum inhibitory concentration determination, and acute oral toxicity testing in mice. At 1000 µg/mL, the n-hexane fraction showed 27.60% inhibition of protein denaturation, while ethyl acetate and indomethacin showed 26.60% and 27.10%, respectively. The n-butanol fraction produced 39.66% inhibition of heat-induced haemolysis, whereas n-hexane produced 25.60% inhibition of hypotonic haemolysis. These are numerical observations because inferential statistical testing was not performed. The aqueous fraction produced the largest observed antimicrobial zone (18 mm against Candida albicans at 200 mg/mL). No mortality occurred at oral doses up to 5000 mg/kg. The findings indicate polarity-dependent differences in the measured responses and support further chromatographic standardization, controlled replication and mechanism-based evaluation.

Keywords: Lophira lanceolata; Ochnaceae; solvent partitioning; membrane stabilization; protein denaturation; antimicrobial activity

Introduction

Inflammation is a protective response to infection and tissue injury, but persistent or dysregulated inflammation contributes to chronic disease (Germolec *et al.*., 2018; Karin *et al.*., 2006). Non-steroidal anti-inflammatory drugs remain useful; however, their prolonged use may be limited by gastrointestinal, renal, and cardiovascular adverse effects (Bhala *et al.*., 2013). In parallel, antimicrobial resistance continues to reduce the effectiveness of established anti-infective agents, reinforcing the need for chemically diverse leads (Salam *et al.*., 2023).
Medicinal plants remain important sources of structurally diverse secondary metabolites.

The extraction solvent and subsequent partitioning influence the classes and concentrations of compounds recovered and may therefore alter the measured biological activity (Altemimi *et al.*., 2017; Alara *et al.*., 2021; Azwanida, 2015). Fraction-level comparisons can provide a practical first step towards identifying active chemical space before bioassay-guided isolation (Sticher, 2008).

Lophira lanceolata Tiegh. ex Keay (Ochnaceae), commonly called dwarf red ironwood, is distributed across tropical West and Central Africa. Different parts of the plant are traditionally used to treat fever, gastrointestinal disorders, pain, and infections (Mapongmetsem, 2007; Sani *et al.*., 2007; Sani *et al.*., 2011). Previous studies have described chalcone dimers, flavonoids, triterpenoids, and other metabolites in Lophira species, together with antioxidant, antiplasmodial, anthelmintic, and antimicrobial activities (Ajiboye *et al.*., 2014; Ali *et al.*., 2011; Collins *et al.*., 2014; Kalmobé *et al.*., 2017; Sinan *et al.*., 2020; Azizi *et al.*., 2023). Although anti-inflammatory activity has been reported for L. lanceolata leaves, comparative evidence for polarity-defined stem-bark fractions remains limited (Ahoton *et al.*., 2023).

This study therefore compared the phytochemical composition, in vitro anti-inflammatory activity, and antimicrobial activity of a methanol stem-bark extract and its n-hexane, ethyl acetate, n-butanol, and aqueous fractions. The acute oral toxicity of the crude extract was also assessed. The study was designed as an exploratory screening investigation to identify fractions that merit further chemical standardization and mechanism-based testing.

Materials and methods

Plant material and authentication

The stem bark of L. lanceolata was collected in January 2025 from Ojoto, Idemili North Local Government Area, Anambra State, Nigeria. The material was authenticated by a taxonomist in the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, and a voucher specimen was deposited under voucher number PCG/474/0/011. The bark was air-dried at room temperature for 14 days and mechanically pulverized.

Extraction and solvent partitioning

Pulverized bark (1000 g) was macerated in 2.5 L of methanol for 72 h with intermittent agitation. The mixture was strained through muslin cloth and filtered through Whatman No. 1 filter paper. The filtrate was concentrated to obtain the crude methanol extract. A portion (54.7 g) of the crude extract was reconstituted in 50 mL of methanol and successively partitioned in a separating funnel with n-hexane, ethyl acetate and n-butanol (500 mL per partition step, repeated twice). The residual polar portion was retained as the aqueous fraction, and all fractions were concentrated by solvent evaporation. Crude-extract yield was calculated as dry extract mass divided by the 1000 g starting plant material, multiplied by 100. Fraction recoveries were calculated relative to the 54.7 g portion of crude extract subjected to partitioning; consequently, crude-extract yield and fraction recoveries are reported on different denominators.

Qualitative phytochemical screening

The crude extract and solvent fractions were screened using standard colour and precipitation tests for alkaloids, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, carbohydrates, proteins, saponins, and reducing sugars. Wagner's, ferric chloride, aluminium chloride, Molisch's, Millon's, and Fehling's tests were used as appropriate (Altemimi *et al.*., 2017; Tiwari *et al.*., 2011).

Acute oral toxicity

Acute oral toxicity of the crude extract was evaluated in mice using the two-phase

method of Lorke (1983). In phase I, groups of three mice received 10, 100 or 1000 mg/kg orally. In phase II, one mouse per dose received 1600, 2900 or 5000 mg/kg. One untreated mouse served as a concurrent control. Animals were observed during the first 4 h, at 24 h and subsequently for 14 days for visible signs of toxicity and mortality. When no death occurred at 5000 mg/kg, the median lethal dose was reported as greater than 5000 mg/kg rather than calculated.

Animal ethics

The acute toxicity protocol was approved by the Animal Research Ethics Committee, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (PHACOOU/AREC/2025/008).

Human participants

Blood was obtained from a healthy adult volunteer after written informed consent. The protocol was approved by the Human Research Ethics Committee, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (PHACOOU/AHEC/2025/005) and conducted in accordance with the Declaration of Helsinki.

In vitro anti-inflammatory assays

The crude extract, fractions and indomethacin were tested at 125, 250, 500 and 1000 µg/mL. Protein-denaturation inhibition was determined by incubating the protein preparation in buffered medium with each test sample, followed by heat-induced denaturation and spectrophotometric measurement against a reagent blank. A heated protein preparation without test sample served as the negative control, and indomethacin served as the reference drug. Percentage inhibition was calculated as [(Acontrol − Asample)/Acontrol] × 100. Results were summarized descriptively.

For erythrocyte membrane stabilization, blood from a healthy adult volunteer who had not used an anti-inflammatory medicine during the preceding 10 days was collected into an EDTA tube after written informed consent and approval by the Human Research Ethics Committee, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (PHACOOU/AHEC/2025/005). Erythrocytes were separated at 3000 rpm for 10 min, washed three times with isotonic saline and reconstituted as a 40% v/v suspension in pH 7.4 phosphate-buffered saline. Aliquots were exposed either to 54 °C for 20 min or to hypotonic medium at room temperature. After centrifugation at 2000 rpm for 5 min, haemoglobin release was measured at 540 nm. Percentage inhibition of haemolysis was calculated as [(Acontrol − Asample)/Acontrol] × 100 against the corresponding heated or hypotonic control (Ikpeazu *et al.*.., 2021; Eze *et al.*., 2019). Results were summarized descriptively.

Antimicrobial assay

The crude extract and fractions were tested against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and a dermatophyte reported as Tinea spp. Inocula were adjusted to 0.5 McFarland turbidity and spread uniformly on appropriate agar media. Extract concentrations of 12.5–200 mg/mL were introduced into 6-mm wells. The plates were incubated under conditions appropriate for each organism. Inhibition was recorded as the net clear zone beyond the edge of each well. Ciprofloxacin (5.6 mg/mL) and fluconazole (4.8 mg/mL) served as antibacterial and antifungal positive controls, respectively.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using a two-fold dilution series of 200, 100, 50, 25 and 12.5 mg/mL. Microbial inocula were standardized to 0.5 McFarland turbidity, exposed to each concentration and incubated under conditions

appropriate for each organism. The MIC was recorded as the lowest tested concentration showing no visible growth. Results were interpreted descriptively; EUCAST clinical breakpoints were not applied because they are not validated for crude plant extracts or fractions (European Committee on Antimicrobial Susceptibility Testing, 2022). MIC findings were considered alongside agar-well diffusion zones because diffusion is influenced by solubility and migration through agar.

Data presentation

Assay results were summarized descriptively. Comparisons were based on observed values, and no inferential statistical testing was performed.

Results

Extraction yield and phytochemical profile

The crude methanol extract yielded 5.74% relative to the starting plant material. Recoveries of the n-hexane, ethyl acetate and n-butanol fractions were 1.34%, 3.25% and 4.30%, respectively, relative to the 54.7 g crude-extract portion partitioned. Alkaloids and tannins were detected in the crude, n-butanol and ethyl acetate samples, whereas terpenoids and proteins were detected in all samples examined. Flavonoids were detected in the n-hexane, n-butanol and ethyl acetate fractions but not in the crude extract (Table 1). This qualitative pattern may reflect enrichment during partitioning, matrix interference or differences in test sensitivity.

Table 1. Qualitative phytochemical profile of *L. lanceolata* stem-bark extract and fractions

Constituent	Crude methanol	n-Hexane	n-Butanol	Ethyl acetate
Alkaloids	+	−	+	+
Tannins	+	−	+	+
Flavonoids	−	+	+	+
Steroids	−	−	−	−
Terpenoids	+	+	+	+
Cardiac glycosides	−	−	−	−
Carbohydrates	−	−	−	−
Proteins	+	+	+	+
Saponins	−	−	−	−
Reducing sugars	+	−	+	+

Key: +, detected; −, not detected by the qualitative test.

Acute oral toxicity

No visible signs of toxicity or mortality were recorded at any tested dose, from 10 to 5000 mg/kg, during the observation period. The oral LD_{50} was therefore greater than 5000 mg/kg under the conditions of this screening test.

Protein-denaturation and membrane-stabilizing activity

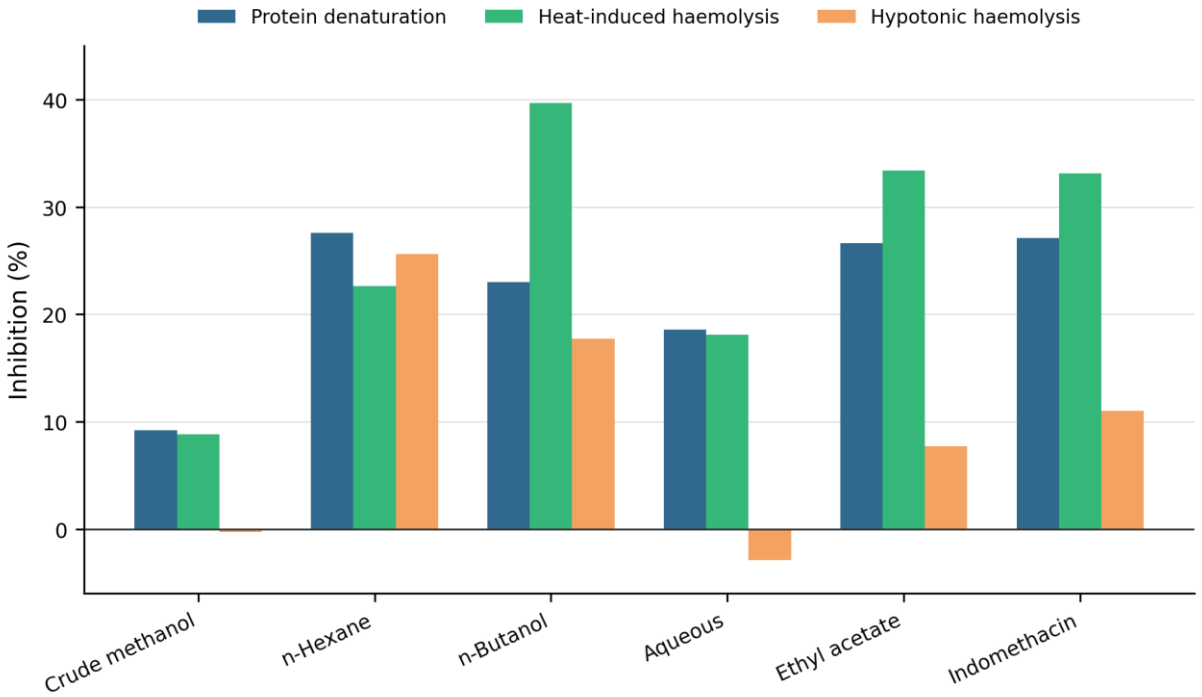
At 1000 µg/mL, the observed protein-denaturation inhibition values were 27.60% for n-hexane, 27.10% for indomethacin and 26.60% for ethyl acetate. The n-butanol fraction showed 39.66% inhibition of heat-induced haemolysis, while n-hexane showed 25.60% inhibition under hypotonic stress (Table 2). These statements describe numerical values only; no statistically significant differences can be inferred. Negative values at some lower concentrations indicate haemolysis greater than that of the assay control and do not represent protection.

Table 2. In vitro anti-inflammatory responses at 1000 µg/mL

Sample	Protein denaturation inhibition (%)	Heat-induced haemolysis inhibition (%)	Hypotonic haemolysis inhibition (%)
Crude methanol extract	9.20	8.84	−0.27
n-Hexane fraction	27.60	22.65	25.60
n-Butanol fraction	23.00	39.66	17.73
Aqueous fraction	18.60	18.11	−2.87
Ethyl acetate fraction	26.60	33.39	7.73
Indomethacin	27.10	33.15	11.00

Values are presented as observed assay responses; statistical significance was not assessed.

Figure 1. Comparative in vitro anti-inflammatory responses of *L. lanceolata* stem-bark extract and fractions at 1000 µg/mL



Negative values indicate haemolysis greater than the assay control and do not represent membrane protection.

Antimicrobial activity

Antimicrobial activity varied according to the solvent fraction and test organism (Table 3). The aqueous fraction produced the largest observed inhibition zone (18 mm against C. albicans at 200 mg/mL) and retained activity at 50 mg/mL (13 mm). The crude extract produced small inhibition zones against *S. aureus*, *B. subtilis, E. coli*, and *Tinea spp.* The ethyl acetate fraction was most active against B. subtilis (10 mm at 200 mg/mL), whereas the n-hexane and n-butanol fractions showed weaker and more selective effects. None of the fractions inhibited *P. aeruginosa*, except the n-hexane fraction at 200 mg/mL (5 mm).

Table 3a. Antimicrobial activity of the crude extract against the test organisms

	Test organisms / Inhibition zone diameter (mm)

Extract concentration (mg/mL)	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*Tinea spp*
200	4±0	5±0	6±0	0±0	0±0	4±0
100	3±0	4±0	5±0	0±0	0±0	0±0
50	0±0	0±0	0±0	0±0	0±0	0±0
25	0±0	0±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0
Pos. Ctrl	0	12	7	8	20	9

Key: S. aureus, Staphylococcus aureus; B. subtilis, Bacillus subtilis; E. coli, Escherichia coli; P. aeruginosa, Pseudomonas aeruginosa; C. albicans, Candida albicans; Tinea spp., dermatophyte reported as Tinea species; Pos. Ctrl, positive control. Ciprofloxacin (5.6 mg/mL) and fluconazole (4.8 mg/mL) were used as the antibacterial and antifungal positive controls, respectively. Values represent net clear zones measured beyond the edge of the 6-mm well.

Table 3b. Antimicrobial activity of the aqueous fraction against the test organisms

Extract concentration (mg/mL)	Test organisms / Inhibition zone diameter (mm)					
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*Tinea spp*
200	5±0	10±0	7±0	0±0	18±0	6±0
100	0±0	9.5±0.7	5±0	0±0	17.5±0.7	0±0
50	0±0	8±0	0±0	0±0	13±0	0±0
25	0±0	7±0	0±0	0±0	0±0	0±0
12.5	0±0	6.5±0.7	0±0	0±0	0±0	0±0
Pos. Ctrl	0	12	7	8	20	9

Table 3c. Antimicrobial activity of the n-butanol fraction against the test organisms

Extract concentration (mg/mL)	Test organisms / Inhibition zone diameter (mm)					
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*Tinea spp*
200	6±0	6±0	5±0	0±0	8±0	0±0
100	0±0	4±0	0±0	0±0	0±0	0±0
50	0±0	0±0	0±0	0±0	0±0	0±0
25	0±0	0±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0
Pos. Ctrl	0	12	7	8	20	9

Table 3d. Antimicrobial activity of the ethyl acetate fraction against the test organisms

Extract concentration (mg/mL)	Test organisms / Inhibition zone diameter (mm)					
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*Tinea spp*
200	0±0	10±0	5±0	0±0	6±0	0±0
100	0±0	9.5±0.7	0±0	0±0	5.5±0.7	0±0
50	0±0	9±0	0±0	0±0	5±0	0±0
25	0±0	4±0	0±0	0±0	4.5±0.7	0±0
12.5	0±0	3.5±0.7	0±0	0±0	0±0	0±0
Pos. Ctrl	0	12	7	8	20	9

Table 3e. Antimicrobial activity of the n-hexane fraction against the test organisms

Extract concentration (mg/mL)	Test organisms / Inhibition zone diameter (mm)					
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***	***C. albicans***	***Tinea spp***
200	6±0	6±0	7±0	5±0	0±0	0±0
100	0±0	5±0	3±0	0±0	0±0	0±0
50	0±0	4±0	0±0	0±0	0±0	0±0
25	0±0	0±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0
Pos. Ctrl	0	12	7	8	20	9

Table 4. Minimum inhibitory concentrations against the test organisms

Extract	Test organisms / minimum inhibitory concentration (mg/mL)					
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***	***C. albicans***	***Tinea spp***
Crude	200	100	100	>200	>200	200
Aqueous	200	12.5	100	>200	50	200
Butanol	200	100	200	>200	200	>200
Ethyl acetate	>200	12.5	200	>200	25	>200
n-hexane	200	50	200	200	>200	>200

Discussion

The findings indicate that solvent partitioning redistributed detectable phytochemical classes and produced different numerical bioactivity profiles. At 1000 µg/mL, n-hexane, ethyl acetate and indomethacin gave closely grouped observed protein-denaturation values (27.60%, 26.60% and 27.10%, respectively), while n-butanol and ethyl acetate showed higher numerical heat-induced membrane-stabilization values than the crude extract. Because inferential statistics were not performed, these patterns should be treated as descriptive rather than evidence of equivalence, superiority or significant fraction effects. Solvent-dependent recovery of plant constituents and corresponding differences in measured activity are consistent with previous reports (Altemimi *et al.*., 2017; Alara *et al.*., 2021; Azwanida, 2015; Sticher, 2008). The qualitative detection of flavonoids in several fractions but not in the crude extract may reflect enrichment, matrix interference or test sensitivity and requires repeat testing before mechanistic interpretation.

The numerical protein-denaturation response of n-hexane was slightly above that of indomethacin, while ethyl acetate was slightly below it at the highest tested concentration. The n-butanol fraction produced the highest observed heat-induced membrane-stabilization value, and n-hexane produced the highest observed value under hypotonic stress. These numerical rankings do not establish statistical differences. Tannins, flavonoids and terpenoids may interact with proteins or membranes through several physicochemical mechanisms (Tiwari *et al.*., 2011; Saxena *et al.*., 2013), but the present screening data do not identify the responsible constituents. Negative inhibition values at lower concentrations indicate absence of protection under those conditions and argue against a uniformly concentration-dependent response.

The extract and fractions showed selective and generally weak-to-moderate antimicrobial responses that varied by organism and solvent fraction (Tables 3a–e). The aqueous fraction produced the largest observed net clear zone, 18 mm against C. albicans at 200 mg/mL; ethyl acetate showed

its largest bacterial response against B. subtilis. Several extract–organism combinations showed no measurable activity. These observations do not support a claim of uniform broad-spectrum activity. MIC values ranged from 12.5 mg/mL to >200 mg/mL. Earlier studies reported antimicrobial effects of L. lanceolata leaves (Sani *et al.*., 2011; Sinan *et al.*., 2020), but direct potency comparison is limited by differences in extract composition and assay design. Diffusion-zone data are influenced by solubility and agar migration and do not distinguish bacteriostatic from bactericidal effects. Further work should include minimum bactericidal or fungicidal concentration determination and controlled repeat assays.

The absence of mortality at 5000 mg/kg suggests a low hazard following a single oral exposure, but it does not establish long-term safety. Repeated-dose studies and assessments of organ function are needed before therapeutic safety can be inferred (Lorke, 1983; Parasuraman, 2011).

Conclusion

Solvent partitioning of the L. lanceolata stem-bark extract produced distinct observed response profiles. At 1000 µg/mL, n-hexane, ethyl acetate and indomethacin showed protein-denaturation inhibition values of 27.60%, 26.60% and 27.10%, respectively; n-butanol showed the highest numerical heat-induced haemolysis inhibition, and n-hexane showed the highest numerical hypotonic-haemolysis inhibition. The aqueous fraction produced the largest observed antifungal zone against C. albicans. No mortality occurred following a single oral dose up to 5000 mg/kg. The findings are exploratory and do not establish comparative superiority, therapeutic efficacy or long-term safety.

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Conflict of interest

The authors declare no conflict of interest.

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