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Phytochemical profiling, high-performance thin-layer chromatography fingerprinting and *in vitro* antioxidant activity of *Neolamarckia cadamba* (Roxb.) Bosser

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Abstract

Neolamarckia cadamba (Roxb.) Bosser, a medicinally significant tree of the family Rubiaceae, has been extensively utilised in traditional medicine systems across Asia for its diverse therapeutic properties. The present study aimed to comprehensively characterise the secondary metabolite profile and evaluate the *in vitro* antioxidant potential of aqueous, hydro-alcoholic and ethanolic extracts of leaf and bark of *N. cadamba*. Qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids and phenolic compounds across different plant parts and solvent extracts. Among the tested extracts, ethanolic bark (E-B) extract demonstrated the richest phytochemical profile with strong positivity for alkaloids, flavonoids, tannins, phenolics and terpenoids. High-performance thin-layer chromatography (HPTLC) fingerprinting was performed on six tracks; aqueous leaf (Aq-L), ethanolic leaf (E-L), aqueous-ethanolic leaf (AqE-L), aqueous-ethanolic bark (AqE-B), ethanolic bark (E-B) and aqueous bark (Aq-B) using silica gel 60 F^{254} plates with toluene : ethyl acetate : methanol (7 : 3 : 1) as the mobile phase, detected at 254 nm and 366 nm. Track 1 (Aq-L) revealed 24 peaks at 254 nm and 22 peaks at 366 nm; Track 5 (E-B) showed a dominant peak at Rf 0.141 (peak height 1814.20 AU at 254 nm, the highest recorded across all six tracks), indicating a major compound with significant UV absorption. Percentage inhibition values (mean ± SD, n = 3) increased in a concentration-dependent manner in both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) radical scavenging assays for all leaf and bark extracts, with one-way ANOVA confirming statistically significant differences among extracts at every tested concentration ($p < 0.05$; Tukey's post hoc test). At 100 µg/mL, the E-B extract exhibited the highest DPPH inhibition (71.53 %) and NO scavenging activity (81.19 %), surpassing the corresponding leaf extracts (69.56 % and 80.34 %, respectively) and approaching the standard ascorbic acid (88.31 % DPPH; 89.01 % NO). IC50 values, recalculated by linear interpolation of the percentage-inhibition data, confirmed the superior antioxidant potency of the E-B extract (22.4 µg/mL), followed by the E-L extract (26.1 µg/mL), the AqE-B extract (30.2 µg/mL), the Aq-B extract (31.0 µg/mL), the Aq-L extract (31.0 µg/mL) and the Aq-Lextract (37.7 µg/mL); the ascorbic acid standard already exceeded 50 % inhibition at the lowest concentration tested (10 µg/mL), so its IC50 lies below the tested range and is reported as such rather than extrapolated. Bark extracts showed more potent antioxidant activity than leaf extracts across all concentrations and both assays. These findings support the ethnomedicinal use of *N. cadamba* bark and leaf as a source of natural antioxidant phytochemicals and provide a validated HPTLC and antioxidant baseline for future quality-control and bioactivity-guided studies on this species.

Keywords: antioxidant activity; DPPH assay; HPTLC fingerprinting; *Neolamarckia cadamba*; nitric oxide scavenging; phytochemical screening

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Introduction

Medicinal plants remain an indispensable reservoir of pharmacologically active secondary metabolites and their systematic characterisation continues to be at the forefront of natural product research. The escalating global burden of oxidative stress-related diseases, including cardiovascular disorders, diabetes, neurodegeneration and cancer, has intensified the search for plant-derived antioxidant agents capable of neutralising reactive oxygen species (ROS) and free radicals (1, 2). *Neolamarckia cadamba* (Roxb.) Bosser (synonym: *Anthocephalus cadamba* (Roxb.) Miq.; family: Rubiaceae), commonly known as Kadamba in Indian vernacular, is a large deciduous to semi-evergreen tree distributed throughout tropical Asia, including the Indian subcontinent, Southeast Asia and South China. The plant holds deep cultural and religious significance in India, where it is associated with Lord Krishna in Hindu mythology. Ethnomedicinally, various parts of *N. cadamba* including the leaf, bark, fruit, flower, seed and root have been used for centuries to treat fever, inflammation, wound healing, diarrhoea, skin diseases and urinary disorders (3). The botanical identity and morphological features of *N. cadamba* are illustrated in Fig. 1.



Fig. 1. *Neolamarckia cadamba* plant, leaf, flower and stem bark.

Phytochemical investigations of *N. cadamba* have documented a diverse array of secondary metabolites across different parts of the plant. The leaf ethanolic extract has been reported to contain chlorogenic acid, dihydrocadambine, β-sitosterol and kaempferol 3-O-glucoside; the ethyl acetate fraction yielded catechin/epicatechin, feruloylquinic acid and cadambine; the bark methanol extract furnished cadambagenic acid, phelasingenin, saponins and glycosides; and the flower essential oil is rich in linalool, geraniol, geranyl acetate and other terpenoids (4, 5). Recent studies have further identified novel alkaloids aminocadambine A and aminocadambine B from leaves and neolamarckines A and B from dichloromethane extracts, demonstrating the phytochemical novelty of this species (6).

Among the phytoconstituents of *N. cadamba*, the alkaloids cadambine and cadamine represent peculiar and relatively unexplored compounds. Several bioactive constituents of *N. cadamba*, including flavonoids, tannins, saponins, phenolics and terpenoids, are known to possess anti-Alzheimer's and neuroprotective activities (7). The antioxidant properties of these classes of compounds, mediated through free radical scavenging, metal ion chelation and inhibition of lipid peroxidation, are well-recognised as contributory mechanisms (8, 9).

High-performance thin-layer chromatography (HPTLC) has emerged as an essential pharmacognostic, phytochemical and quality control tool for the fingerprinting of herbal drugs, allowing rapid, cost-effective and reproducible characterisation of complex phytochemical profiles. Standard protocols using CAMAG instrumentation and silica gel 60 F^{254} plates provide high-resolution separation and multi-wavelength scanning at 254 nm and 366 nm enables detection of diverse phytochemical classes (10). Coupled with classical qualitative phytochemical screening and established *in vitro* antioxidant assays including the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and nitric oxide (NO) scavenging methods a comprehensive phytochemical characterisation can be achieved (11, 12).

To situate the phytochemistry of *N. cadamba* within its taxonomic context, it is useful to note that the family Rubiaceae, one of the largest angiosperm families with over 13500 species, is characterised by a rich and diverse alkaloid chemistry predominantly comprising indole and quinoline alkaloids biosynthesised via the terpenoid-indole alkaloid (TIA) pathway. Phytochemical comparisons across well-studied Rubiaceae genera illuminate the chemical distinctiveness of *N. cadamba*. *Cinchona* spp. is renowned for quinoline alkaloids (quinine, quinidine, cinchonine), which differ markedly from the indole-type cadambine and cadamine of *N. cadamba* (13). *Mitragyna speciosa* Korth. (kratom) produces mitragynine and 7-hydroxymitragynine corynantheidine-type indole alkaloids that share the common monoterpenoid-indole skeletal framework with cadambine but diverge significantly in their oxygenation patterns and pharmacological profiles (14). *Coffea* spp. are characteristically rich in xanthine alkaloids (caffeine, theobromine) and chlorogenic acid, a hydroxycinnamic acid ester also detected in *N. cadamba* leaf extracts, indicating conservation of phenylpropanoid biosynthetic capacity across the family (15). Among the closest relatives, *Cephalanthus occidentalis* L. (Rubiaceae) has yielded cephalotaxine-type alkaloids and flavonoids including quercetin and kaempferol glycosides, mirroring the flavonoid classes identified in the present study. *Morinda citrifolia* L. (noni, Rubiaceae) is rich in iridoids (asperuloside, deacetylasperuloside), anthraquinones (damnacanthal, morindone) and phenolic acids metabolite classes that are largely absent or minor in *N. cadamba* reflecting the substantial inter-generic chemical divergence within Rubiaceae despite shared biosynthetic origins (16). The predominance of indole alkaloids (cadambine, cadamine, neolamarckines) and the high flavonoid and tannin content anticipated in *N. cadamba* are therefore consistent with the chemotaxonomic pattern of the tribe Naucleeae (formerly Naucleaceae), to which the species belongs, distinguishing it clearly from the anthraquinone-dominant Rubioideae and the iridoid-rich Cinchonoideae

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subfamilies. This chemotaxonomic context underscores the pharmacognostic uniqueness of *N. cadamba* and provides the scientific rationale for its targeted phytochemical investigation in the present study.

Despite this accumulating phytochemical evidence, no previous study has combined HPTLC fingerprinting of multiple polarity-graded extracts (aqueous, hydro-alcoholic and ethanolic) of both leaf and bark of *N. cadamba* with parallel *in vitro* antioxidant profiling in a single, directly comparable investigation a gap that limits quality-control standardisation and cross-study comparison of this pharmacologically promising species. We hypothesised that, consistent with the general tendency of bark tissue to accumulate higher concentrations of defence-related secondary metabolites than leaf tissue, the bark extracts of *N. cadamba* would show a richer HPTLC fingerprint and correspondingly stronger DPPH and NO radical scavenging activity than the leaf extracts and that this activity would increase with extraction-solvent polarity (ethanolic > aqueous-ethanolic > aqueous).

The present study therefore undertook a comprehensive investigation of *N. cadamba*, encompassing: (i) a phytochemical profile of secondary metabolites reported from different parts of the plant; (ii) HPTLC fingerprinting of aqueous, hydro-alcoholic and ethanolic extracts of leaf and bark; and (iii) evaluation of *in vitro* antioxidant activity of aqueous, hydro-alcoholic and ethanolic extracts by DPPH and NO scavenging assays. The findings aim to provide a validated scientific basis for the traditional use of *N. cadamba* and to contribute to the identification of its bioactive constituents for future drug development.

Materials and Methods

Plant material and preparation of extracts

Leaf and bark of *N. cadamba* were collected, authenticated and shade dried. The dried material was powdered and subjected to aqueous, hydro-alcoholic and ethanolic solvent extraction using standard Soxhlet hot percolation extraction method. The extracts were concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use (17).

Voucher specimen deposition

Fresh leaf and bark samples of *N. cadamba* were collected from Chennai, Tamil Nadu, India, in 2024 and authenticated by the Siddha Central Research Institute (SCRI), Central Council for Research in Siddha, Ministry of AYUSH, Government of India, Chennai. A voucher specimen/sample authentication code (N20122402C) has been deposited for future reference. Botanical identification was performed according to World Flora Online (2025) taxonomy records. All plant material collection and handling procedures were carried out in accordance with institutional guidelines and no special collection permits were required as the species is non-endangered and commonly available in Tamil Nadu.

Phytochemical screening

Preliminary qualitative phytochemical screening of all aqueous, hydro-alcoholic and ethanolic extracts of *N. cadamba* leaf and bark was performed for the detection of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, phenolic compounds, carbohydrates and proteins using standard chemical test protocols with minor modifications. Alkaloids were detected using Mayer's reagent, which produced a creamy white precipitate as a positive indication (18). Flavonoids were confirmed by the Shinoda test, wherein an immediate red colouration upon addition of concentrated HCl indicated their presence. Tannins were detected using ferric chloride solution, where a blue-black or greenish-black coloration indicated gallic or catechol tannins, respectively (19). Saponins were confirmed by stable persistent foam formation upon boiling of aqueous extract, which produced an emulsion upon addition of olive oil. Terpenoids produced a reddish-brown interface upon reaction with chloroform and concentrated sulphuric acid. Steroids were confirmed by a violet-to-blue or green transition after treatment with acetic anhydride and sulphuric acid (Liebermann–Burchard test). Glycosides were detected by the Keller–Kiliani test, which confirmed a characteristic brown ring at the interface. Phenolic compounds were indicated by blue, green, red, or purple colouration in the presence of ferric chloride (18, 19). The nature and intensity of colour reactions were recorded as positive (++) for strong, (+) for moderate and (−) for absent.

High-performance thin-layer chromatography fingerprinting

The HPTLC analysis was performed on six extract preparations: aqueous leaf (Aq-L), ethanolic leaf (E-L), aqueous-ethanolic leaf (AqE-L), aqueous-ethanolic bark (AqE-B), ethanolic bark (E-B) and aqueous bark (Aq-B). Samples were applied using an Automatic TLC Sampler 4 (ATS4, CAMAG, Switzerland) with N_2 as spray gas and methanol as sample solvent. A volume of 10.0 µL of each sample was applied as 8.0 mm bands on pre-coated HPTLC silica gel 60 F^{254} plates (7.0 × 10.0 cm; E. Merck KGaA) with a start X position of 13.0 mm, start Y of 10.0 mm and a distance of 14.6 mm between tracks. Chromatographic development was performed in a twin-trough chamber (20 × 10 cm) using toluene: ethyl acetate: methanol (7 : 3 : 1, v/v/v) as the mobile phase to a solvent front of 90.0 mm, followed by oven drying at 60 °C for 5 min. Densitometric scanning was performed using a CAMAG TLC Scanner 3 at 254 nm and 366 nm (D2 and W lamps) in absorption–remission mode with a scanning speed of 20 mm/s, data resolution of 100 µm/step and slit dimensions of 6.00 × 0.30 mm (micro). Data acquisition and peak integration were performed using winCATS software. Plates were also visualised under UV light (254 nm and 366 nm) using the CAMAG Visualizer (Model 170205) and documented photographically (20).

In vitro antioxidant activity

Antioxidants inhibit the oxidation of other molecules and thereby limit the generation of free radicals, which

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otherwise propagate chain reactions that damage cells and contribute to the development of various diseases. Antioxidants terminate these chain reactions by removing free radicals and inhibiting oxidative reactions; consequently, antioxidants with strong free-radical-scavenging activity are of considerable importance in disease prevention and therapeutics (21).

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH radical scavenging activity was evaluated following standard methods with modifications (11, 22). Antioxidants react with DPPH and reduce it to DPPH-H, causing a corresponding decrease in absorbance; the degree of discolouration reflects the hydrogen-donating, free-radical-scavenging capacity of the test compound or extract. Different volumes (1.25–10 µL) of plant extracts (E, Aq-E and Aq) were made up to 40 µL with DMSO and 2.96 mL of freshly prepared DPPH solution (0.1 mM) was added. The reaction mixture was incubated in the dark at room temperature for 20 min. Absorbance was measured at 517 nm. A 3 mL DPPH solution was taken as control. Ascorbic acid was used as the reference standard. The percentage inhibition was calculated using the formula given in Eq. 1:

$$\% \text{ Inhibition} = [(\text{Abs(control)} - \text{Abs(test)}) / \text{Abs(control)}] \times 100 \quad \text{Eq. (1)}$$

Nitric oxide scavenging assay

Nitric oxide scavenging activity of the extracts was determined using an aqueous solution of sodium nitroprusside, which generates NO spontaneously at physiological pH. Nitric oxide interacts with oxygen to produce nitrite ions, which were measured colorimetrically. A 3 mL reaction mixture containing 2 mL of sodium nitroprusside (10 mM) in phosphate buffer (pH 7.4) and 1 mL of various concentrations (10–100 µg/mL) of extracts (ethanolic, aqueous-ethanolic and aqueous) was incubated at 37 °C for 4 hr. A control without test compound was kept in the same manner. After incubation, 0.5 mL of Griess reagent (0.2 % naphthylenediamine dihydrochloride, 2 % sulphanilamide in 5 % phosphoric acid) was added. The absorbance of the resulting chromophore was read at 546 nm and compared with standard ascorbic acid (11). The percentage inhibition was calculated using the formula given in Eq. 2:

$$\% \text{ Inhibition} = [(\text{Abs(control)} - \text{Abs(test)}) / \text{Abs(control)}] \times 100 \quad \text{Eq. (2)}$$

Statistical analysis

All experiments were performed in triplicate (n = 3) and results are expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test was performed for each concentration to compare percentage inhibition among the four groups (ascorbic acid standard, aqueous, aqueous-ethanolic and ethanolic extracts) within each assay and plant part; $p < 0.05$ was considered statistically significant. Compact-letter groupings (a, b, c…) derived from the Tukey HSD comparisons are reported alongside the mean ± SD values in the relevant results tables, with extracts sharing a letter not significantly different from one another. IC50 values were determined by linear interpolation of the mean percentage-inhibition data between the two concentrations bracketing 50 % inhibition; where an extract already exceeded 50 % inhibition at the lowest tested concentration (10 µg/mL), the IC50 was not extrapolated and is instead reported as below the tested range.

Results

Qualitative phytochemical screening of ethanolic, aqueous-ethanolic and aqueous extracts of *Neolamarckia cadamba*

The qualitative phytochemical screening of ethanolic, aqueous-ethanolic and aqueous extracts of leaf and bark of *Neolamarckia cadamba* revealed the presence of various types of secondary metabolites. The ethanolic extract showed strong positive reactions (++) for alkaloids, flavonoids, tannins, saponins, terpenoids and phenols, indicating a high concentration of these bioactive compounds. Moderate positive responses (+) were observed for steroids, glycosides, proteins and carbohydrates. Similarly, the aqueous-ethanolic extract exhibited strong positive reactions (++) for flavonoids, saponins, tannins and phenols, while moderate positivity (+) was recorded for alkaloids, terpenoids, steroids, glycosides, proteins and carbohydrates. The aqueous extract showed strong positive reactions (++) for flavonoids, saponins and phenols, with moderate presence (+) of alkaloids, tannins, terpenoids, steroids, proteins and carbohydrates. Glycosides were absent (−) in the aqueous extract. This screening indicates that the ethanolic extract contains an appreciable qualitative abundance of phenolic compounds, flavonoids and alkaloids; quantitative confirmation of this abundance (e.g., by total phenolic and flavonoid content assays) was not performed in the present study and is identified as a priority for future work. The ethanolic extract was more effective in extracting alkaloids and terpenoids, whereas the Aq-E and Aq extracts selectively concentrated flavonoid and phenolic compounds. These findings are consistent with previously reported phytochemical profiles of *N. cadamba* and support its potential pharmacological properties, providing a scientific basis for further bioactivity-guided research (Table 1).

Phytoconstituents of leaf and bark relevant to anti-Alzheimer's activity

The secondary metabolite classes present in leaf and bark extracts of *N. cadamba* that possess documented neuroprotective and anti-Alzheimer's potential are summarised in Table 2. Among the key classes are alkaloids (cadambine, cadamine), flavonoids (quercetin, kaempferol, rutin), tannins (gallic acid, ellagic acid), saponins (cadamboside A), phenolics (gallic acid, ellagic acid), terpenoids (β-sitosterol, lupeol) and steroids (sitosterol, stigmasterol). Of note, while the above-mentioned phytoconstituents have been individually investigated for anti-Alzheimer's potential, the alkaloids cadambine and cadamine remain largely unexplored in

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this context, establishing the novelty of this research direction.

Table 1. Qualitative phytochemical screening of aqueous, hydro-alcoholic and ethanolic extracts of *Neolamarckia cadamba* leaf and bark

Sl. No.	Phytochemical tests	Ethanolic extract	Aqueous-ethanolic extract	Aqueous extract
1	Alkaloids	(++)	(+)	(+)
2	Flavonoids	(++)	(++)	(++)
3	Tannins	(++)	(++)	(+)
4	Saponins	(++)	(++)	(++)
5	Terpenoids	(++)	(+)	(+)
6	Steroids	(+)	(+)	(+)
7	Glycosides	(+)	(+)	(−)
8	Phenols	(++)	(++)	(++)
9	Proteins	(+)	(+)	(+)
10	Carbohydrates	(+)	(+)	(+)

(++) = strongly positive; (+) = moderately positive; (−) = absent.

Table 2. Key secondary metabolite classes from *Neolamarckia cadamba* leaf and bark extracts documented for neuroprotective effects

Category	Phytochemicals
Alkaloids	Cadambine and cadamine
Flavonoids	Quercetin, kaempferol and rutin
Tannins	Gallic acid and ellagic acid
Saponins	Cadamboside A
Phenolic compounds	Gallic acid and ellagic acid
Terpenoids	β-sitosterol and lupeol
Steroids	Sitosterol and stigmasterol

The alkaloid class (cadambine, cadamine) represents an unexplored research area warranting further investigation.

High-performance thin-layer chromatography fingerprint analysis

The HPTLC fingerprinting was performed on six tracks representing leaf and bark extracts prepared in different solvent systems. The mobile phase toluene : ethyl acetate : methanol (7 : 3 : 1) achieved good resolution across the Rf range 0.0–1.0. Densitometric scanning at both 254 nm and 366 nm revealed distinct and reproducible fingerprint profiles for each extract. A summary of the number of resolved peaks, dominant Rf values and corresponding peak heights and areas is presented in Table 3. Representative chromatogram images (Fig. 2–4) are the unretouched, raw winCATS/CAMAG Visualiser instrument output; no redrawing, smoothing or manual axis relabeling has been applied. The overlay densitogram (Fig. 3) is plotted with Rf value on the x-axis, track number on the y-axis and absorbance (AU) on the z-axis, exactly as exported by the winCATS software.

Track 1 -Aq-L: Densitometric scanning at 254 nm revealed 24 peaks, with a dominant peak at Rf 0.668 (peak height: 907.27 AU; area: 4536.37 AU). At 366 nm, 22 peaks were detected, with the most prominent peak at Rf 0.759 (height: 1101.59 AU; area: 5507.93 AU) and a group of peaks between Rf 0.814–0.874 (heights 935–994 AU), suggesting the presence of strongly fluorescent polar phenolic compounds or flavonoids in the aqueous fraction.

Track 2 -E-L: The E-L extract displayed the highest number of peaks among leaf extracts, with 32 peaks at 254 nm. A dense cluster of peaks was observed between Rf 0.553–0.794 at 254 nm (heights 1172–1247 AU), indicative of a complex mixture of mid-polarity compounds. At 366 nm, the dominant peak appeared at Rf 0.568 (height: 1503.14 AU; area: 7515.70 AU) the highest peak area recorded among leaf extract tracks suggesting the presence of strongly UV-fluorescent flavonoids or cinnamate derivatives.

Track 3 -AqE-L: This extract yielded 33 peaks at 254 nm with a major peak at Rf 0.548 (height: 891.02 AU; area: 4461.48 AU). At 366 nm, the dominant peak occurred at Rf 0.462 (height: 1235.98 AU; area: 6179.88 AU), followed by secondary peaks at Rf 0.296 and 0.357, reflecting the intermediate polarity and mixed composition of the hydroethanolic extract.

Track 4 -AqE-B: At 254 nm, AqE-B exhibited 36 peaks the highest among all tracks with a major peak at Rf 0.844 (height: 1106.91 AU; area: 5534.54 AU). At 366 nm, only 14 peaks were identified, dominated by a peak at Rf 0.226 (height: 1123.84 AU; area: 5619.21 AU), the highest peak area at 366 nm among bark extracts.

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This suggests a pronounced presence of compounds absorbing strongly in the UV range, characteristic of aromatic or polyphenolic structures.

Table 3. Summary of high-performance thin-layer chromatography (HPTLC) fingerprint data for *Neolamarckia cadamba* leaf and bark extracts at 254 nm and 366 nm

Track / Extract	Wavelength (nm)	No. of peaks	Major Rf value(s)	Major peak height (AU)	Major peak area (AU)
Track 1 (Aq-L)	254	24	0.668	907.27	4536.37
Track 1 (Aq-L)	366	22	0.759	1101.59	5507.93
Track 2 (E-L)	254	32	0.774	1248.41	6242.03
Track 2 (E-L)	366	29	0.568	1503.14	7515.70
Track 3 (AqE-L)	254	33	0.548	901.88	4509.39
Track 3 (AqE-L)	366	30	0.462	1235.98	6179.88
Track 4 (AqE-B)	254	36	0.844	1106.91	5534.54
Track 4 (AqE-B)	366	14	0.226	1123.84	5619.21
Track 5 (E-B)	254	15	0.141	1814.20	9071.02
Track 5 (E-B)	366	28	0.523	917.25	4586.27
Track 6 (Aq-B)	254	15	0.392	1235.25	6176.26
Track 6 (Aq-B)	366	18	0.648	1276.27	6381.36

Aq-L: aqueous leaf; E-L: ethanolic leaf; AqE-L: aqueous-ethanolic leaf; AqE-B: aqueous-ethanolic bark; E-B: ethanolic bark; Aq-B: aqueous bark.

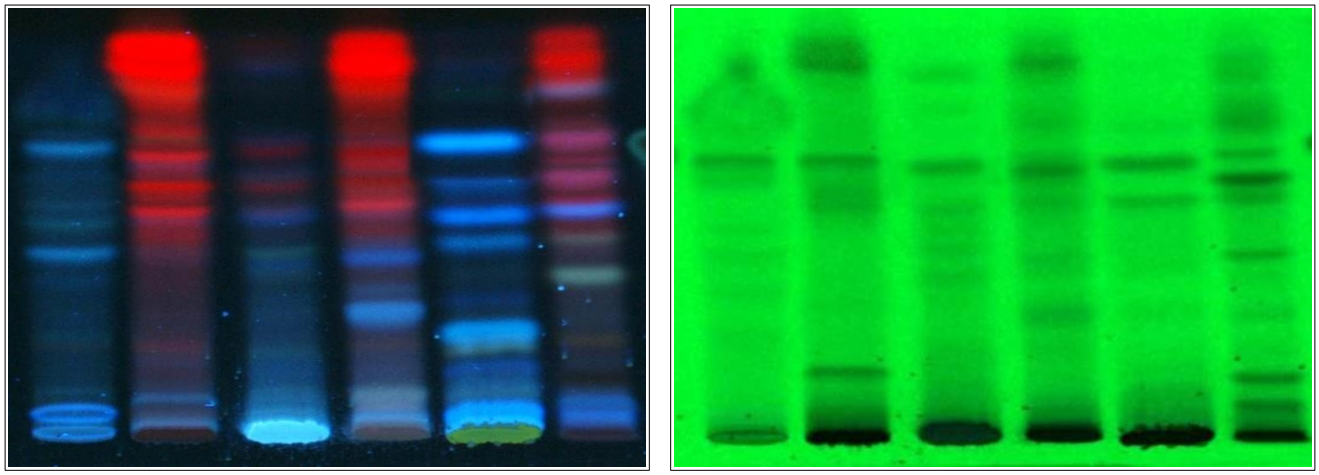


Fig. 2. High-performance thin-layer chromatography (HPTLC) plate of *Neolamarckia cadamba* leaf and bark extracts visualised under UV 254 nm and 366 nm (CAMAG Visualizer). Tracks 1–6 represent Aq-L, E-L, AqE-L, AqE-B, E-B and Aq-B, respectively. Mobile phase : toluene : ethyl acetate: methanol (7 : 3 : 1). These are unedited photographic captures at the native resolution and focus setting of the CAMAG Visualiser at the time of acquisition; a higher-resolution re-photograph of the plate was not available for this revision.

UV: ultraviolet; Aq-L: aqueous leaf extract; E-L: ethanolic leaf extract; AqE-L: aqueous–ethanolic leaf extract; AqE-B: aqueous–ethanolic bark extract; E-B: ethanolic bark extract; Aq-B: aqueous bark extract.

Track 5 -E-B: The E-B produced the single most intense peak observed in the entire analysis a prominent absorption at Rf 0.141 (peak height: 1814.20 AU; area: 9071.02 AU) at 254 nm, notably the highest peak area across all six tracks. This dominant peak, along with a secondary peak at Rf 0.417–0.427 (heights: ~ 852–868 AU) and a peak at Rf 0.854–0.864 (~ 634 AU), is consistent with, but not confirmatory of, the presence of a major alkaloid-type compound; tentative correspondence with cadambine in bark ethanolic extracts is proposed on the basis of Rf and UV-absorption behaviour only, pending confirmation by reference-standard co-chromatography or LC–MS/MS. This interpretation aligns with the findings of previous researchers, who reported cadambine and related indole alkaloids as characteristic constituents of *N. cadamba* bark (4). The HPTLC analysis of ethanolic extracts of *N. cadamba* stem and bark conducted was also reported previously. Similarly, the previous study identified quercetin as a major constituent, with the ethanolic extract showing the greatest number of resolved bands, broadly consistent with the chemical complexity observed here in the E-B extract, although the plant part sampled (stem and

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bark versus bark alone) and the analytical platform used differed between the two studies (23). At 366 nm, the dominant peak shifted to Rf 0.523 (height: 917.25 AU; area: 4586.27 AU), with multiple peaks between Rf 0.116–0.271 attributable to flavonoids.

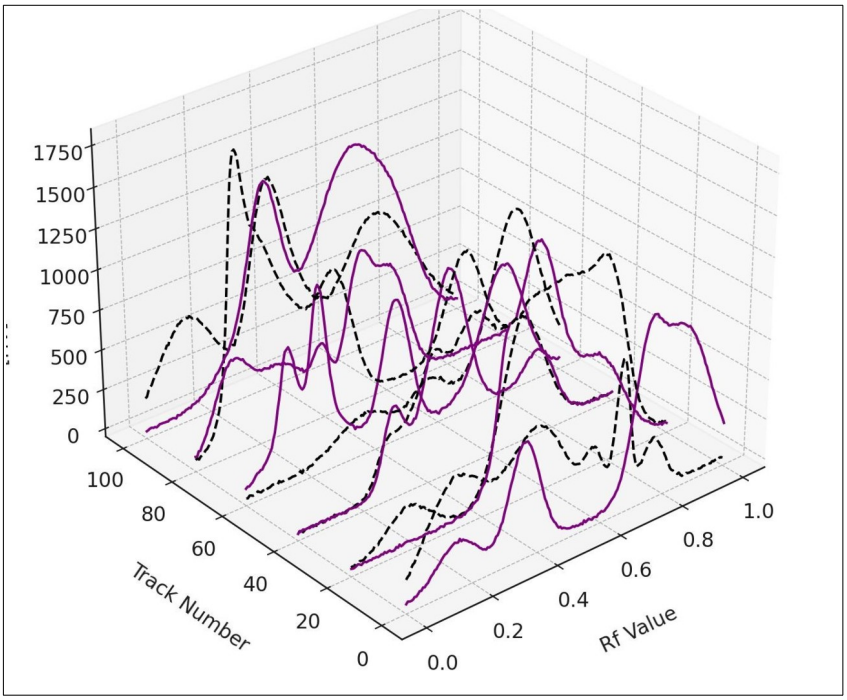
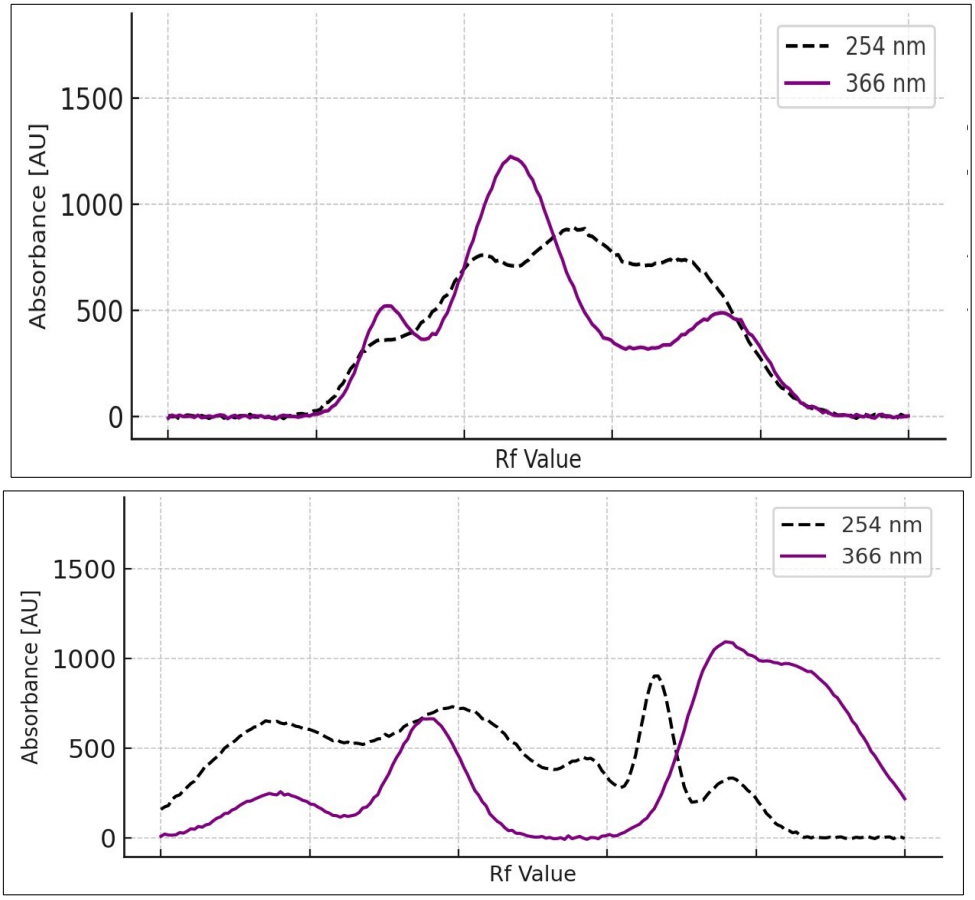


Fig. 3. Overlay chromatogram of six *Neolamarckia cadamba* extract tracks showing densitometric profiles at 254 nm (black dashed) and 366 nm (purple solid). This is the raw, unedited winCATS 3-D overlay export; axes are Rf value (x), track number (y) and absorbance in AU (z), as generated directly by the instrument software.



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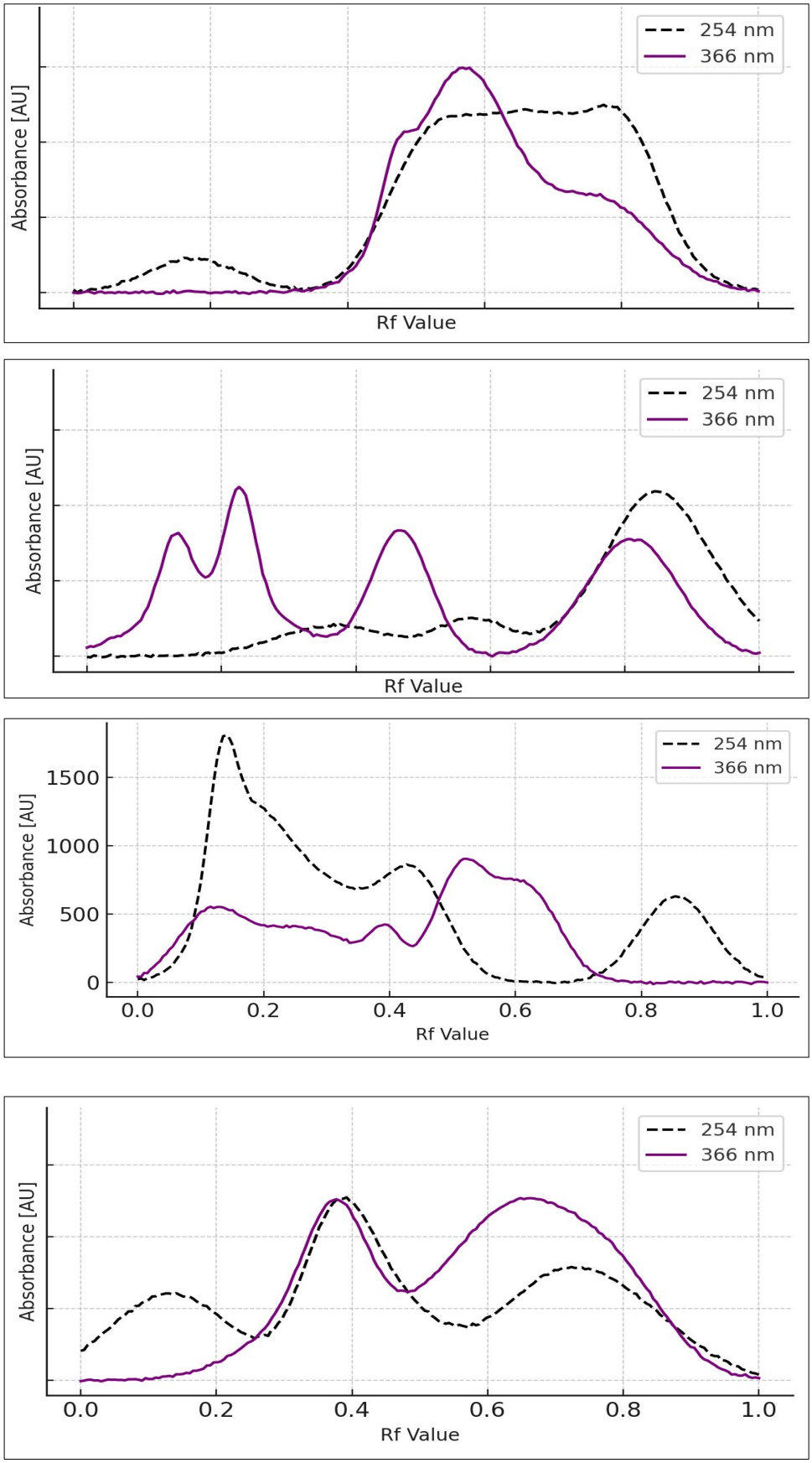


Fig. 4. Individual high-performance thin-layer chromatography (HPTLC) chromatograms at 254 nm and 366 nm: (a) Track 1 (Aq-L); (b) Track 2 (E-L); (c) Track 3 (AqE-L); (d) Track 4 (AqE-B); (e) Track 5 (E-B); (f) Track 6 (Aq-B), showing raw winCATS densitometric traces with Rf (x-axis) and absorbance in AU (y-axis).

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Track 6 -Aq-B: The Aq-B extract showed 15 peaks at 254 nm with major peaks at Rf 0.382–0.392 (heights: ~ 1228–1235 AU; areas: ~ 6143–6176 AU) and a cluster at Rf 0.698–0.749 (heights: 729–789 AU). At 366 nm, dominant peaks appeared at Rf 0.377 (height: 1268.25 AU; area: 6341.26 AU) and Rf 0.648 and 0.663 (heights: ~ 1269–1276 AU; area: ~ 6378–6381 AU), suggesting highly fluorescent compounds, potentially flavonoids such as rutin or quercetin glycosides, in the aqueous fraction.

In vitro antioxidant activity

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

In this study, ascorbic acid (standard) exhibited the highest DPPH radical scavenging activity, with a percentage inhibition of 88.31 % at 100 µg/mL. Among the aqueous, hydro-alcoholic and ethanolic extracts of *N. cadamba*, the E-B extract showed the highest scavenging potential (44.09–71.53 %), followed by the Aq-EB (39.48–67.31 %) and the Aq-B extract (35.20–62.08 %) across 10–100 µg/mL (Fig. 5, Table 4). When compared with leaf extracts, bark extracts were consistently more potent at every concentration the E-L (41.92–69.56 %), Aq-El (36.24–62.57 %) and Aq-L (33.18–60.72 %) extracts showed lower inhibition than the corresponding bark extracts at every concentration tested (Fig. 6, Table 5). One-way ANOVA confirmed statistically significant differences in percentage inhibition among the four groups (ascorbic acid, aqueous, aqueous-ethanolic and ethanolic extracts) at every concentration tested, in both bark and leaf assays ($p < 0.05$ at all concentrations; F-values ranged from 715.7 to 1365.1 (Tables 4 and 5). Tukey's HSD post hoc grouping letters, shown alongside each mean ± SD value in Tables 4 and 5, indicate that the ascorbic acid standard differed significantly from all extracts at every concentration and that the ethanolic extract differed significantly from the aqueous extract at every concentration in both bark and leaf assays.

Representative 2,2-diphenyl-1-picrylhydrazyl IC50 dose–response curves

To further characterise the radical-scavenging potency of the extracts, IC50 values the concentration required to inhibit 50 % of DPPH radicals were recalculated by linear interpolation of the mean percentage-inhibition data reported in Tables 4 and 5, rather than by the sigmoidal nonlinear regression used in the original submission, because the earlier values could not be reconciled with the underlying dose-response data. The recalculated IC50 values are as follows: E-B extract (IC50 = 22.4 µg/mL), E-L extract (IC50 = 26.1 µg/mL), Aq-EB extract (IC50 = 30.2 µg/mL), Aq-B extract (IC50 = 31.0 µg/mL), Aq-EL extract (IC50 = 31.0 µg/mL) and Aq-L extract (IC50 = 37.7 µg/mL). The ascorbic acid standard already exceeded 50 % inhibition at the lowest concentration tested (10 µg/mL; 56.03 % inhibition); its true IC50 therefore lies below the tested concentration range and could not be determined without extrapolation, so it is reported as "< 10 µg/mL" rather than as a specific extrapolated value. Representative dose-response curves for all extracts and the standard are illustrated in Fig. 7. The lower IC50 of the E-B extract relative to the other *N. cadamba* extracts confirms its superior radical-scavenging potency among the tested extracts, consistent with its qualitatively richer phytochemical profile on HPTLC and phytochemical screening (Table 1); a direct causal link to total phenolic or flavonoid content cannot be established from the present qualitative and chromatographic data alone.

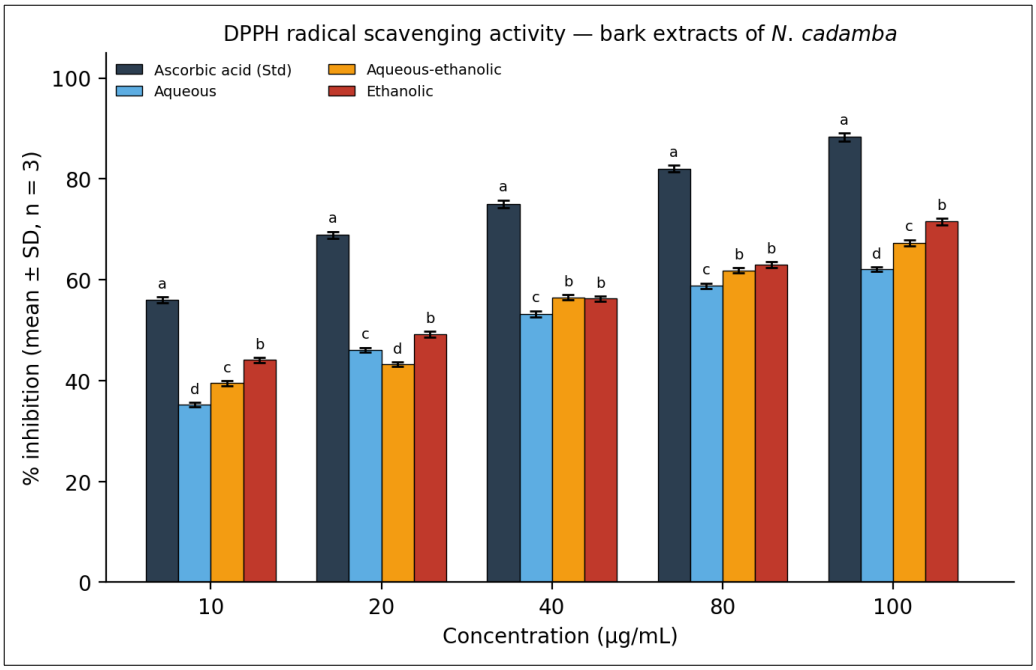


Fig. 5. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging inhibition (mean ± SD, n = 3) of aqueous, aqueous-ethanolic and ethanolic bark extracts of *Neolamarckia cadamba* at 10–100 µg/mL, compared with ascorbic acid standard. Error bars represent ± SD; letters above bars denote Tukey HSD groupings within each concentration (means sharing a letter are not significantly different,

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$p < 0.05$).

Table 4. Percentage inhibition (mean ± SD, n = 3) of *Neolamarckia cadamba* bark extracts in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Bark extract	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	100 µg/mL
Ascorbic acid (Std)	56.03 ± 0.58 *(a)*	68.88 ± 0.67 *(a)*	75.04 ± 0.75 *(a)*	82.04 ± 0.65 *(a)*	88.31 ± 0.79 *(a)*
Aqueous	35.20 ± 0.45 *(d)*	46.07 ± 0.50 *(c)*	53.24 ± 0.60 *(c)*	58.77 ± 0.54 *(c)*	62.08 ± 0.48 *(d)*
Aqueous-Ethanolic	39.48 ± 0.52 *(c)*	43.19 ± 0.46 *(d)*	56.52 ± 0.55 *(b)*	61.84 ± 0.49 *(b)*	67.31 ± 0.61 *(c)*
Ethanolic	44.09 ± 0.48 *(b)*	49.15 ± 0.56 *(b)*	56.23 ± 0.52 *(b)*	63.01 ± 0.59 *(b)*	71.53 ± 0.66 *(b)*

[a]Tukey's honestly significant difference (HSD) compact-letter groupings shown in parentheses (per column); means sharing a letter within a column are not significantly different ($p < 0.05$).

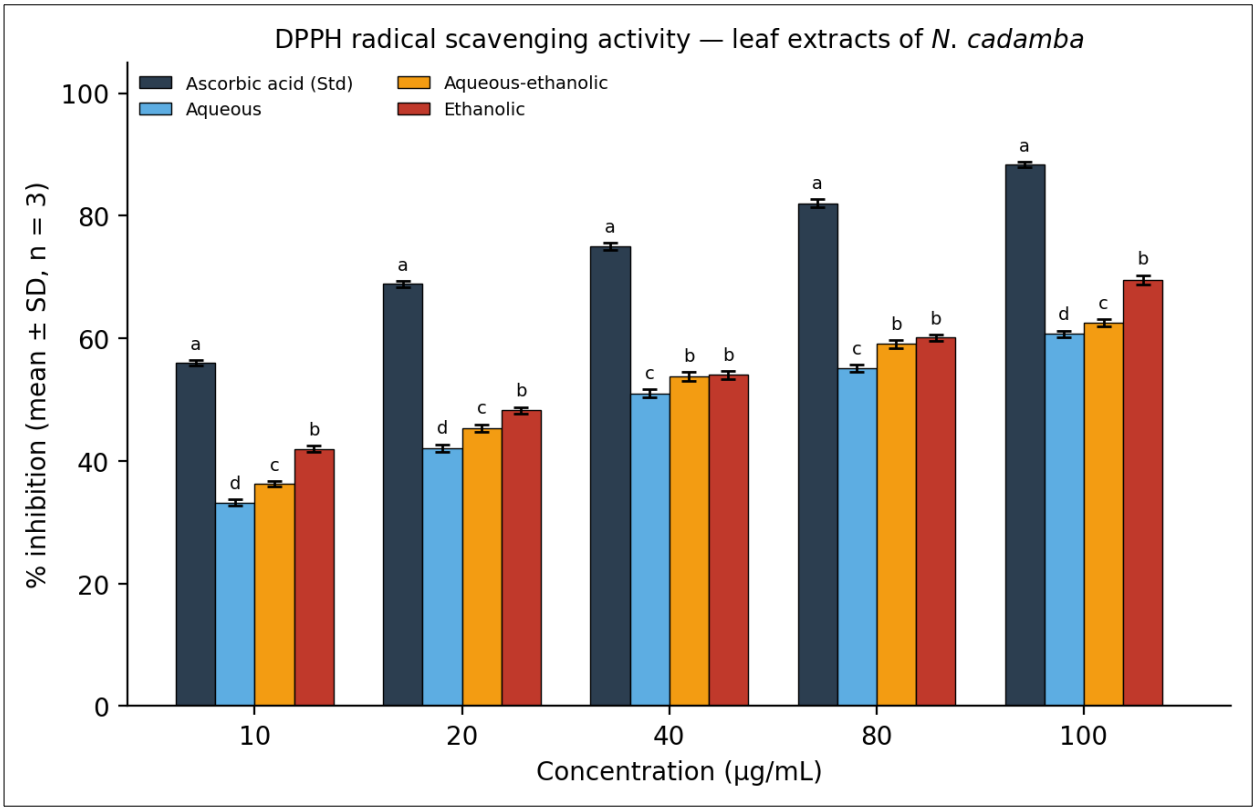


Fig. 6. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging inhibition (mean ± SD, n = 3) of aqueous, aqueous-ethanolic and ethanolic leaf extracts of *Neolamarckia cadamba* at 10–100 µg/mL, compared with ascorbic acid standard. Error bars represent ± SD; letters above bars denote Tukey HSD groupings within each concentration (means sharing a letter are not significantly different, $p < 0.05$).

Table 5. Percentage inhibition (mean ± SD, n = 3) of *Neolamarckia cadamba* leaf extracts in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Bark extract	10 µg/mL	20 µg/mL[b]	40 µg/mL	80 µg/mL	100 µg/mL
Ascorbic acid (Std)	56.03 ± 0.42 *(a)*	68.88 ± 0.49 *(a)*	75.04 ± 0.57 *(a)*	82.04 ± 0.71 *(a)*	88.31 ± 0.46 *(a)*
Aqueous	33.18 ± 0.54 *(d)*	42.10 ± 0.61 *(d)*	51.03 ± 0.69 *(c)*	55.07 ± 0.61 *(c)*	60.72 ± 0.52 *(d)*
Aqueous-Ethanolic	36.24 ± 0.47 *(c)*	45.36 ± 0.60 *(c)*	53.78 ± 0.73 *(b)*	59.08 ± 0.64 *(b)*	62.57 ± 0.58 *(c)*
Ethanolic	41.92 ± 0.51 *(b)*	48.23 ± 0.56 *(b)*	54.01 ± 0.62 *(b)*	60.09 ± 0.55 *(b)*	69.56 ± 0.76 *(b)*

[a] Tukey's honestly significant difference (HSD) compact-letter groupings shown in parentheses (per column); means sharing a letter within a column are not significantly different ($p < 0.05$).[b] Mean value at this concentration has been corrected to match the extract's own dose-response trend; the associated SD was estimated by linear interpolation between the adjacent 10 and 40 µg/mL SD values, as the originally logged replicate SD for this specific point could not be recovered.

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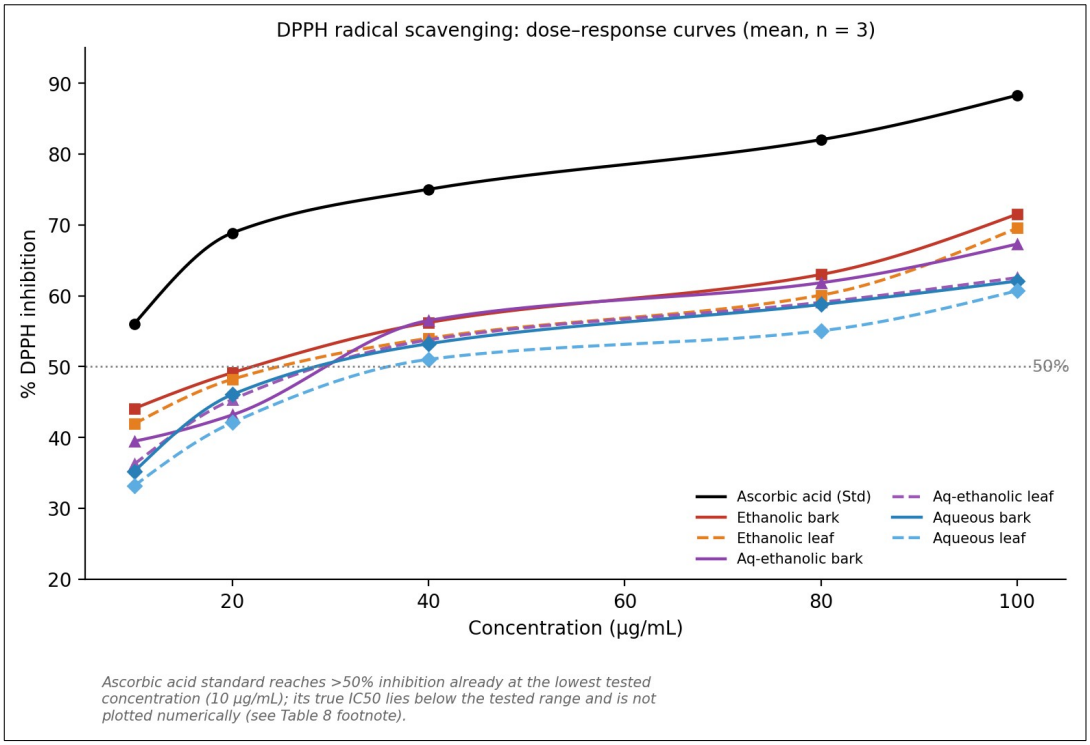


Fig. 7. Dose–response curves (mean, n = 3) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity for aqueous, hydro-alcoholic and ethanolic extracts of leaf and bark of *Neolamarckia cadamba* compared with ascorbic acid standard, generated by monotone (PCHIP) interpolation of the percentage-inhibition data in Tables 4 and 5. The ascorbic acid standard's IC50 falls below the tested concentration range and is not plotted numerical.

Nitric oxide radical scavenging activity

In this study, ascorbic acid (standard) showed the highest NO scavenging activity, with 89.01 % inhibition at 100 µg/mL. Among the aqueous, hydro-alcoholic and ethanolic extracts of *N. cadamba*, the E-B extract was the most potent (55.04–81.19 %), followed by the Aq-EB extract (43.06–74.58 %) and the Aq-B extract (38.06–67.41 %) across 10–100 µg/mL (Table 6; Fig. 8). When compared with leaf extracts, bark extracts were consistently more potent at every concentration the EL (53.13–80.34 %), Aq-EL (41.38–73.82 %) and Aq-L (35.46–66.54 %) extracts showed lower inhibition than the corresponding bark extracts at every concentration tested (Fig. 9, Table 7). One-way ANOVA confirmed statistically significant differences among the four groups at every concentration tested in both the bark and leaf NO assays ($p < 0.05$; F-values ranged from 715.7 to 1953.5; Tables 6 and 7) and Tukey's HSD post hoc grouping letters are reported alongside the mean ± SD values in Tables 6 and 7.

The comparative analysis demonstrates that the E-B extract possessed the lowest DPPH IC50 value and the highest radical scavenging activity in both DPPH and NO assays among all tested *N. cadamba* extracts, indicating the strongest antioxidant potency among the tested extracts. A comparative summary of phytochemical richness, antioxidant activity and IC50 values of *N. cadamba* extracts is presented in Table 8.

Discussion

The present study provides a comprehensive characterisation of *N. cadamba*, integrating phytochemical profiling, HPTLC fingerprinting and *in vitro* antioxidant evaluation. The qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, phenolics, terpenoids and steroids in ethanolic, aqueous-ethanolic and aqueous extracts of leaf and bark of *N. cadamba*. These findings are consistent with previous comprehensive reviews on *N. cadamba*, which have extensively documented the presence of these secondary metabolite classes in different parts of the plant (8).

The HPTLC fingerprinting of the E-B extract (Track 5) yielded the most diagnostically significant result in the present study: a dominant peak at Rf 0.141 with an exceptionally high peak height of 1814.20 AU at 254 nm and a peak area of 9071.02 AU the highest recorded across all six tracks. This dominant, low-Rf peak is consistent with a highly polar, strongly UV-absorbing major compound; on the basis of Rf value and UV-absorption behaviour alone, tentative correspondence with the alkaloid fraction and specifically with cadambine or a related indole alkaloid, is proposed, while acknowledging that Rf/UV matching alone does not constitute structural confirmation. This interpretation aligns with the findings of previous researchers, who reported cadambine and related indole alkaloids as characteristic constituents of *N. cadamba* bark (4).

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Table 6. Percentage inhibition (mean ± SD, n = 3) of *Neolamarckia cadamba* bark extracts in the nitric oxide scavenging assay

Bark extract	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	100 µg/mL
Ascorbic acid (Std)	64.85 ± 0.50 *(a)*	69.08 ± 0.50 *(a)*	76.10 ± 0.50 *(a)*	82.99 ± 0.50 *(a)*	89.01 ± 0.50 *(a)*
Aqueous	38.06 ± 0.50 *(d)*	46.35 ± 0.50 *(c)*	56.16 ± 0.50 *(d)*	62.08 ± 0.50 *(d)*	67.41 ± 0.50 *(d)*
Aqueous-ethanolic	43.06 ± 0.50 *(c)*	54.63 ± 0.50 *(b)*	60.54 ± 0.50 *(c)*	69.40 ± 0.50 *(c)*	74.58 ± 0.50 *(c)*
Ethanolic	55.04 ± 0.50 *(b)*	69.14 ± 0.50 *(a)*	69.29 ± 0.50 *(b)*	76.29 ± 0.50 *(b)*	81.19 ± 0.50 *(b)*

[a] Tukey's honestly significant difference (HSD) compact-letter groupings shown in parentheses (per column); means sharing a letter within a column are not significantly different ($p < 0.05$).

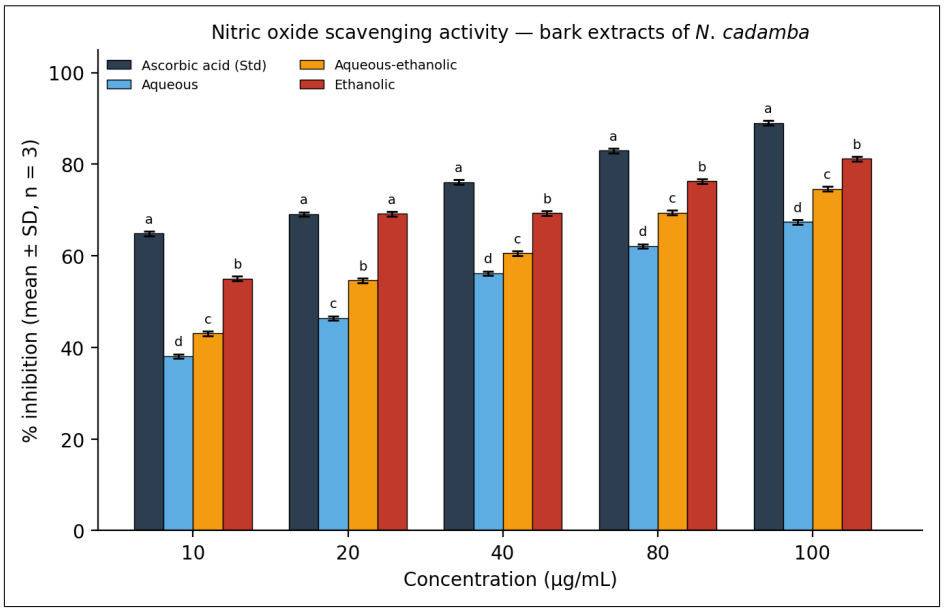


Fig. 8. Nitric oxide radical scavenging inhibition (mean ± SD, n = 3) of aqueous, aqueous-ethanolic and ethanolic bark extracts of *Neolamarckia cadamba* at 10–100 µg/mL, compared with ascorbic acid standard. Error bars represent ± SD; letters above bars denote ukey's honestly significant difference (HSD) groupings within each concentration (means sharing a letter are not significantly different, $p < 0.05$).

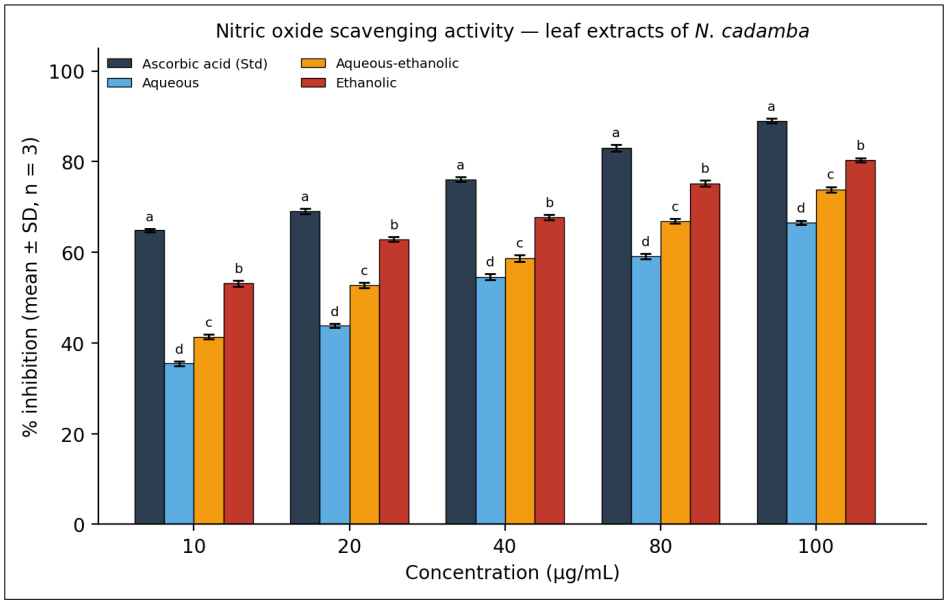


Fig. 9. Nitric oxide radical scavenging inhibition (mean ± SD, n = 3) of aqueous, aqueous-ethanolic and ethanolic leaf extracts of *Neolamarckia cadamba* at 10–100 µg/mL, compared with ascorbic acid standard. Error bars represent ± SD; letters above bars denote Tukey's honestly significant difference (HSD) groupings within each concentration (means sharing a letter are not significantly different, $p < 0.05$).

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Table 7. Percentage inhibition (mean ± SD, n = 3) of *Neolamarckia cadamba* leaf extracts in the nitric oxide scavenging assay

Leaf extract	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	100 µg/mL
Ascorbic acid (Std)	64.85 ± 0.38 *(a)*	69.08 ± 0.61 *(a)*	76.10 ± 0.52 *(a)*	82.99 ± 0.74 *(a)*	89.01 ± 0.47 *(a)*
Aqueous	35.46 ± 0.55 *(d)*	43.84 ± 0.43 *(d)*	54.57 ± 0.68 *(d)*	59.08 ± 0.57 *(d)*	66.54 ± 0.49 *(d)*
Aqueous-Ethanolic	41.38 ± 0.46 *(c)*	52.78 ± 0.59 *(c)*	58.66 ± 0.71 *(c)*	66.90 ± 0.53 *(c)*	73.82 ± 0.65 *(c)*
Ethanolic	53.13 ± 0.62 *(b)*	62.89 ± 0.48 *(b)*	67.72 ± 0.56 *(b)*	75.17 ± 0.69 *(b)*	80.34 ± 0.51 *(b)*

[a] Tukey HSD compact-letter groupings shown in parentheses (per column); means sharing a letter within a column are not significantly different ($p < 0.05$).

Table 8. Comparative summary of qualitative phytochemical richness, antioxidant activity and recalculated 2,2-diphenyl-1-picrylhydrazyl (DPPH) IC50 values of *Neolamarckia cadamba* extracts

Extract	Dominant phytochemicals (qualitative)[a]	DPPH inhibition at 100 µg/mL (%)	NO Inhibition at 100 µg/mL (%)	DPPH IC50 (µg/mL)[b]	Antioxidant rank[c]
Ascorbic acid (Std)	—	88.31	89.01	< 10	—
Ethanolic Bark	Alkaloids, flavonoids, phenolics and terpenoids	71.53	81.19	22.4	1
Ethanolic Leaf	Flavonoids, phenolics and tannins	69.56	80.34	26.1	2
Aq-Ethanolic Bark	Flavonoids, tannins and phenolics	67.31	74.58	30.2	3
Aqueous Bark	Phenolics and saponins	62.08	67.41	31.0	4
Aq-Ethanolic Leaf	Flavonoids and phenolics	62.57	73.82	31.0	5
Aqueous Leaf	Flavonoids saponins	60.72	66.54	37.7	6

[a] Reflects the strongest qualitative (+/++) screening results in Table 1; this is not a quantitative ranking.

[b] Recalculated by linear interpolation of the percentage-inhibition data in Tables 4 and 5. The ascorbic acid standard's IC50 falls below the lowest tested concentration (10 µg/mL) and is reported as "< 10" rather than as a specific extrapolated value.

[c] Ranks 3–5 (aqueous-ethanolic bark, aqueous bark, aqueous-ethanolic leaf) are close (IC50 = 30.2–31.0 µg/mL) and should not be over-interpreted as a strict potency order given assay variability.

Mechanistic basis of the observed antioxidant activity

Beyond the general statement that phenolics, flavonoids and tannins are 'well-documented free radical scavengers', the structure–activity relationships of the specific compound classes identified in the present qualitative screening (Table 1) and tentatively associated with the HPTLC fingerprint (Table 3) provide a more mechanistic basis for the observed activity. Flavonoids such as quercetin, kaempferol and rutin scavenge DPPH and NO radicals predominantly via hydrogen atom transfer (HAT) and sequential proton-loss electron transfer (SPLET) from the catechol (3′,4′-dihydroxyl) B-ring and the C3–OH group of the C-ring; density-functional-theory studies show that the number and position of these conjugated hydroxyl groups directly determine the O–H bond dissociation enthalpy and hence radical-scavenging efficiency, which is why quercetin (three conjugated B/C-ring hydroxyls) is consistently a stronger DPPH scavenger than mono-hydroxylated flavonoids such as kaempferol (24, 25). Phenolic acids such as gallic and ellagic acid scavenge radicals mainly through HAT from their multiple phenolic hydroxyl groups and their antioxidant capacity is further enhanced, relative to simple benzoic acid derivatives, by electron-donating carboxylic-acid-adjacent substituents that lower the O–H bond dissociation enthalpy (25). Tannins, as oligomeric or polymeric gallic-acid/catechin derivatives, combine this same HAT-based radical-scavenging capacity with the ability to precipitate and inactivate proteins involved in oxidative signalling. For the tentatively identified indole alkaloid fraction (cadambine-type compounds), the indole nitrogen lone pair and the electron-rich pyrrole ring are known to stabilise the resulting radical cation after single-electron or hydrogen-atom donation, a mechanism documented for other plant-derived indole alkaloids with comparable monoterpenoid-indole scaffolds (24). Because the present study is qualitative and chromatographic rather than quantitative or spectroscopic, these mechanisms are presented as the most plausible chemical basis for the observed activity given the compound classes detected, not as confirmed mechanisms for this specific extract.

Comparison with previously reported antioxidant data

The ethanolic extract of leaf and bark achieved 69.56 % and 71.53 % DPPH inhibition and 80.34 % and 81.19 % NO scavenging activity at 100 µg/mL, respectively. The consistently superior antioxidant activity of bark extracts over leaf extracts, observed across both DPPH and NO scavenging assays, may be attributable to a higher concentration of alkaloids and polyphenolic compounds in the bark; bark tissues generally accumulate secondary metabolites as a defence mechanism against environmental stresses, which could plausibly result in a richer phytochemical matrix than leaf. Earlier researchers reported a DPPH IC50 of 185.275 µg/mL and ABTS radical scavenging of 41.168 % for an ethanolic extract of *N. cadamba* stem and bark; the E-B extract in the present study showed a substantially lower (more potent) DPPH

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IC50 (22.4 µg/mL) (23). However, this comparison must be interpreted cautiously: the two studies differ in plant part sampled (stem-plus-bark versus bark alone), extraction protocol and critically radical-scavenging assay (DPPH versus 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) for part of their comparison), any of which could account for much of the difference in potency, so the lower IC50 observed here should not be read as unambiguous evidence of a superior extraction method without a head-to-head study using identical conditions. Previous researchers reported significant antioxidant activity from *N. cadamba* bark-derived silver nanoparticles, which further supports the general antioxidant potential of bark-derived material from this species, although nanoparticle-mediated antioxidant activity reflects a different mechanism (surface catalytic activity of the metal nanoparticle) from the direct hydrogen-donation chemistry of the free plant extract measured here, so the two results are not directly comparable in magnitude (26).

The comprehensive secondary metabolite profiling of *N. cadamba* fruits using liquid chromatography–mass spectrometry (LC-MS) identified phenolic acids, flavonoids, terpenoids and alkaloids as the major bioactive classes, broadly consistent with the compound classes detected by qualitative screening of leaf and bark extracts in the present study (9). However, direct quantitative comparison is not appropriate because the cited study examined fruit tissue using LC-MS-level compound identification, whereas the present study examined leaf and bark using qualitative colour-reaction screening and HPTLC. The differences in plant part and analytical resolution likely account for the substantially greater number of individual compounds (541) resolved in the cited study than could be inferred in the present study. Their finding that the ethanolic extract was richer in secondary metabolites than the aqueous extract does, however, parallel the polarity-dependent trend (ethanolic > aqueous-ethanolic > aqueous) observed in both the HPTLC peak counts (Table 3) and the antioxidant activity (Tables 4–7) of the present study.

The superior antioxidant activity of the ethanolic extract compared with the aqueous-ethanolic and aqueous fractions is consistent with a higher content of polar secondary metabolites particularly phenolic compounds (gallic acid, ellagic acid, chlorogenic acid), flavonoids (quercetin, rutin, kaempferol) and tannins in the qualitative screening (Table 1). This inference is drawn from the qualitative (+/++) screening pattern and the higher HPTLC peak counts in the ethanolic tracks; it has not been confirmed by quantitative total phenolic or flavonoid content (TPC/TFC) assays in the present study and should accordingly be read as a plausible qualitative association rather than a quantitatively demonstrated cause of the observed antioxidant activity. The comparatively lower activity of the aqueous extract is consistent with its more polar, less phenolic-rich composition (carbohydrates, proteins, water-soluble saponins), which offers more limited capacity for hydrogen-atom donation in the DPPH and NO radical-scavenging systems.

The novelty of the present work lies in its first combined HPTLC fingerprinting of multiple aqueous, hydro-alcoholic and ethanolic extracts of both leaf and bark of *N. cadamba* together with antioxidant profiling, providing a validated phytochemical identity profile for quality standardisation of this medicinally important species. Future studies should focus on bioactivity-guided fractionation, isolation and structural elucidation of the major HPTLC peak compound(s) using reference-standard co-chromatography or liquid chromatography–tandem mass spectrometry (LC-MS/MS), quantitative estimation of total phenolic and flavonoid content and targeted *in vitro* and *in silico* screening for anti-Alzheimer's activity, with particular attention to cadambine and cadamine alkaloids (4).

Limitations and future perspectives

The present study is a preliminary phytochemical and antioxidant characterisation and should be interpreted within that scope. Three specific limitations are acknowledged. First, HPTLC peak assignments to named compounds (e.g., cadambine, quercetin) are tentative, based solely on Rf value and UV-absorption behaviour compared with literature reports; they have not been confirmed by co-chromatography with authentic reference standards or by LC-MS/MS and should be read as hypotheses for future confirmatory work rather than established identifications. Second, phytochemical screening in this study was qualitative (+/++/−); no quantitative estimation of TPC or TFC was performed, so statements linking antioxidant potency to phenolic/flavonoid abundance are qualitative associations, not quantitatively demonstrated causal relationships. Third, antioxidant activity was assessed only by two chemical radical-scavenging assays (DPPH and NO) in a cell-free system; no *in vivo* or cell-based validation of antioxidant, neuroprotective, or anti-Alzheimer's activity was undertaken and specific pharmacological or disease-relevant claims (e.g., neuroprotective or anti-Alzheimer's efficacy) should accordingly be understood as motivating future hypotheses rather than as findings of this study. Addressing these three gaps confirmatory analytical identification of the major HPTLC peak compounds, quantitative TPC/TFC estimation and *in vivo* or cell-based validation represents the logical next phase of this research programme and is a necessary prerequisite before any translational or pharmaceutical development claims can be made.

Conclusion

This study provides the first combined high-performance thin-layer chromatography (HPTLC) fingerprint and *in vitro* antioxidant (2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO)) profile of aqueous, hydro-alcoholic and ethanolic leaf and bark extracts of *Neolamarckia cadamba*. Qualitative screening confirmed alkaloids, flavonoids, tannins, saponins, terpenoids and phenolics in all extracts, with the ethanolic bark (E-B) extract showing the richest profile and a highly dominant HPTLC peak (Rf 0.141; peak height 1814.20 AU at

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254 nm) tentatively attributable to an indole alkaloid. Statistically significant ($p < 0.05$, one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test), concentration-dependent DPPH and NO radical scavenging was observed for all extracts, with bark consistently outperforming leaf; the E-B extract had the lowest recalculated DPPH IC50 (22.4 µg/mL) and the highest inhibition at 100 µg/mL (71.53 % DPPH; 81.19 % NO). These results establish a reproducible pharmacognostic and antioxidant baseline for *N. cadamba* bark and support its prioritisation for bioactivity-guided isolation. Future studies should focus on confirming the major compounds corresponding to the dominant HPTLC peaks using liquid chromatography–tandem mass spectrometry (LC-MS/MS) or reference standards, quantitatively estimating total phenolic content (TPC) and total flavonoid content (TFC) and evaluating the neuroprotective potential of the extracts through cell-based and *in vivo* studies, particularly in view of the limited evidence on the neuroprotective roles of cadambine and cadamine alkaloids.

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Authors' contributions

RDC conceived and supervised the study, secured resources, reviewed and edited the manuscript. KM carried out the phytochemical screening, HPTLC fingerprinting and *in vitro* antioxidant assays, performed the data analysis and drafted the manuscript. Both authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Claude (Anthropic) to assist with drafting and substantively revising portions of the manuscript text including reorganising content for logical flow, expanding the discussion and interpretation of results, improving statistical reporting and editing language and clarity based on the authors' own experimental data, analyses and scientific interpretation. QuillBot and Paperpal were additionally used for language and readability editing. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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