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Research Article

Integrative Quality standard profiling of Three Kadamba species; *Neolamarkia cadamba* (Roxb.)Bosser, *Adina cordifolia* (Roxb.) Brandis, *Mitragyna parvifolia* (Roxb.) Korth.

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Abstract

About: *Neolamarkia cadamba* (Roxb.) Bosser, *Adina cordifolia* (Roxb.)Brandis, *Mitragyna parvifolia* (Roxb.) Korth are three different tree species belonging to the angiosperm family *Rubiaceae*. These trees species possess immense medicinal properties with splendid phytochemical constituents which are considered the major sources of *Kadamba* in Ayurveda. Objective: To compare and evaluate the three sources of *Kadamba* through performing macroscopic, microscopic, phytochemical, physicochemical, and high-performance thin-layer chromatography (HPTLC) analyses following standard protocols. Materials and Methods: Bark collection of these trees is done in the month of December. After obtaining proper authentication proceeded with the studies according to the standard protocol. Result: Three sources of *Kadamba* show similar bioactive compounds like alkaloids, tannins, glycosides with slightly acidic pH, and high water-soluble extractive values. Despite their similarities, they also exhibit distinct differences in morphology, microscopic characteristics, and specific phytochemical profiles. Conclusion: Detailed profiling of all three samples provides a vital foundation for future research, quality control standards in medicinal use.

Keywords: *Adina cordifolia* (*Roxb.*) *Brandis,* HPTLC, *Kadamba*, *Neolamarkia cadamba* (*Roxb.*) *Bosser, Mitragyna parvifolia* (*Roxb.*) *Korth,* Phytochemical, Physicochemical.

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Introduction

Neolamarkia cadamba (Roxb.), is a fast-growing, large deciduous tree found along riverbanks and in transitional swampy areas. It is Common in the sub-Himalayan region, West Bengal, Bihar, Orissa, Andhra Pradesh, Karnataka, Kerala, and the Western Ghats (1). Prefers wet places and often has a buttressed base (*Adina cordifolia* (Roxb.) large deciduous tree with a crown, growing 18-30 meters tall (2). Found across South and Southeast Asia, including India, Burma, Sri Lanka and Southeast China(3). Tree is known for a straight, clean trunk that can be up to 150 cm in diameter and often buttressed at the base (4). *Mitragyna parvifolia* (Roxb.) Korth is found in deciduous forests, thriving in well-drained, deep soil (5). Distributed across tropical and semi-arid regions of Africa, India, China, Myanmar, Sri Lanka and Southeast Asia belonging to *Rubiaceae* family (6).

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These trees are taken as the major sources for *Kadamba* mentioned in Ayurveda classics, well known for thier aromatic flowers and astringent bark (7). They contain numerous phytochemical constituents like tannins, triterpenoids, phenolic compounds which contribute to its various medicinal properties like antimicrobial, anti-inflammatory, antidiabetic etc(8). In traditional medicine *Kadamba* preparations are used in the management of dysentery, fever, skin diseases and wound healing (9). Because of the existence of multiple botanical sources under the common name *Kadmaba* there is a growing need for pharmacognostic study to differentiate these species and to standardize the genuine source for Ayurveda formulation. Marking quality standards of these three drugs following standard methodologies enhances its authentication and supports the development of evidence-based medicinal applications (10).

Materials and methods

The Drug *Neolamarckia cadamba*(Roxb.)Bosser was collected from the bank of the Payaswini River, Sullia ; *Adina cordifolia* (Roxb.)Brandis from the catchment area of Chandragiri River Sullia, Dakshina Kannada district; and *Mitragyna parvifolia* (Roxb.)Korth from the catchment area of Manali River, Peechi, Kerala. Collections were made from December to January(11).

Confirmation of the species was done at Forest Botany Department KFRI, and is deposited with Accession no. 19368,19369,19370.

Macroscopy

Morphological features of three bark pieces were well observed through field lens and recorded using a camera, later verified by comparing them with descriptions provided in the regional flora for accurate identification (12).

Microscopy

Transverse sections of bark from three drugs were prepared using sharp blade and microtome blade. Sections were stained with 1% safranin to enable clear visualization, mounted in 50% glycerin, and covered with a cover slip. Specimens were observed under the Leica DM 2000 microscope and microscopic examinations were conducted at a magnification of 4 to 100 X. Digital photomicrographs were made with LAS (Leica application suite) software (13).

Physico-chemical standards

Loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, and water-soluble extractive were analyzed as per the established standard protocols (14).

Preliminary phytochemical analysis

The phytochemical screening was performed for all three plant sources using four solvent extracts (chloroform, acetone, methanol, and aqueous), ensuring a systematic evaluation of phytoconstituents across solvent polarities. Extracts were subjected to qualitative phytochemical tests to identify major classes of secondary metabolites (15). Alkaloids were detected using Mayer's, Wagner's, Dragendorff's, and Hager's tests. Carbohydrates and reducing sugars were identified by Molisch's, Fehling's and Benedict's tests. Steroids and terpenoids were detected using Salkowski and Liebermann–Burchard tests based on characteristic color changes. Tannins and phenolic compounds were identified using ferric chloride and lead acetate tests, indicated by blue-black coloration and precipitate formation. Flavonoids were detected using Shinoda and alkaline reagent tests, whereas glycosides were identified using the Keller–Kiliani test. The froth formation test used to detect saponins, while amino acids and proteins were identified using the Ninhydrin and Biuret tests, respectively (16).

High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC analysis was performed using a stationary phase of TLC Silica gel 60 F254 (Merk, 1.05554.0007) coated on 20×10cm aluminum sheets. The mobile phase consisted of Toluene: Ethyl acetate: Formic acid: Methanol in the ratio of 14:10:2:1, and visualization was carried out using iodine as ideal reagent for non-UV absorbing compound. The developed plates were visualized in UV 254, 366, under white light and then derivatized with vanillin sulphuric acid and scanned under UV 254 and 366 nm. Rf, the color of the spots and densitometric scan were recorded (17).

Results

Macroscopy

Neolamarckia cadamba(Roxb.)Bosser shown dark brownish with fracture, vertically grooved, exfoliating in rectangular flakes, blaze yellowish creamy brown edges change to browner on exposure to sunlight. *Adina cordifolia* (Roxb.)Brandis bark was greyish black exfoliating in small patches, blaze was reddish pink with a waxy appearance. *Mitragya parvifolia* (Roxb.)Korth grey bark with smooth, irregular thin scaly exfoliation outwards. Blaze is slightly pink turning red on exposure to sunlight (Figure 1 & 2).

Figure 1: Outer bark of (a) Neolamarckia cadamba (b) Adina cordifolia (c) Mitragyna parvifolia

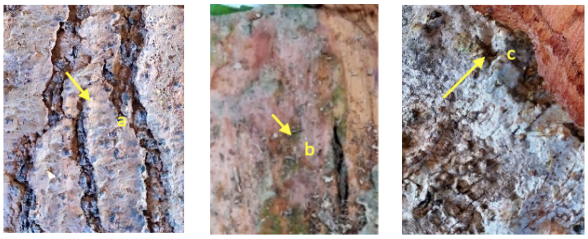
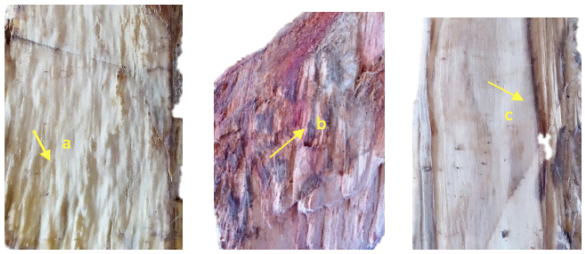


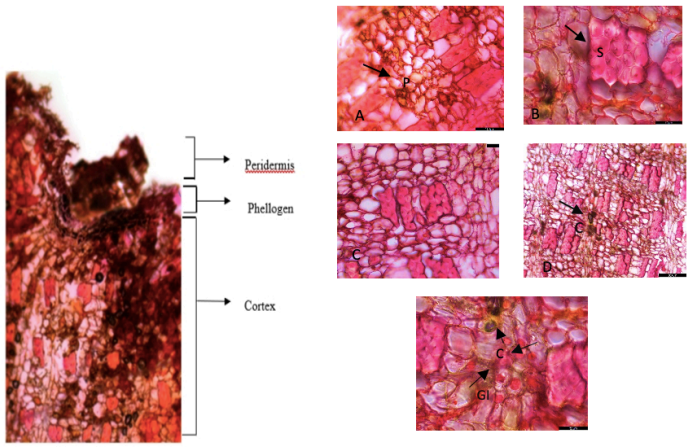
Figure 2: Inner bark (blaze) of (a) *Neolamarckia cadamba* (b) *Adina cordifolia* (c) *Mitragyna parvifolia*



Microscopy

TS of *Neolmarckia cadamba* (Roxb.)Bosser bark show cork cells mainly of stone cells and fibers. Alternate layers of thin-walled, tangentially elongated lignified outermost cells with narrow tangentially running discontinuous bands of lignified stone cells circular to rectangular in shape were found. Cortical tissues consist of layers of irregular, reddish-brown to yellowish-brown parenchymatous cells, occasionally embedded with micro sphenoidal crystals of calcium oxalate, interrupted at places with stone cells. Obliterated parenchymatous cells filled with yellowish brown content & pericyclic band of stone cells were recorded. Phloem had stone cells, medullary rays, phloem fibres, microspenoidal crystals, starch grains. Sieve tissues to multiseriate medullary rays embedded with micro sphenoidal crystals of calcium oxalate and parenchyma with starch grains (Figure 3).

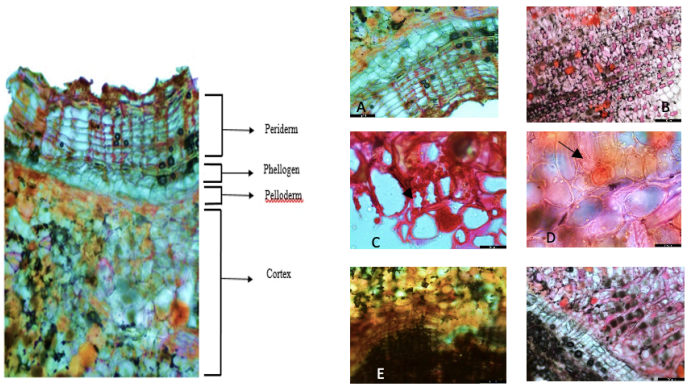
Figure 3: TS bark *Neolamarckia cadamba* (Roxb.) Bosser



A: P Pigment cell; B: S-Stone cells; C: MR Medullary rays; D & E: C Cells with content; GI Granular inclusions.

TS *Adina cordifolia* (Roxb.) Brandis cork show periderm with 6-8 layers, rows of tangentially elongated cells, few cells containing orange colouring mater. Phellogen with 3-4 layers of colourless thin-walled, tangentially elongated cells containing pigments and crystals. Cortex was wide parenchymatous, parenchymal cells of polyhedral shapes with intracellular space, filled with starch, resin, stone cell. Medullary Rays1-2 celled towards the outer bark consisting of thin-walled radially elongated parenchymal cells. Phloem parenchyma encompasses resins, crystals, calcium Oxalate, scattered stone cells. (Figure 4)

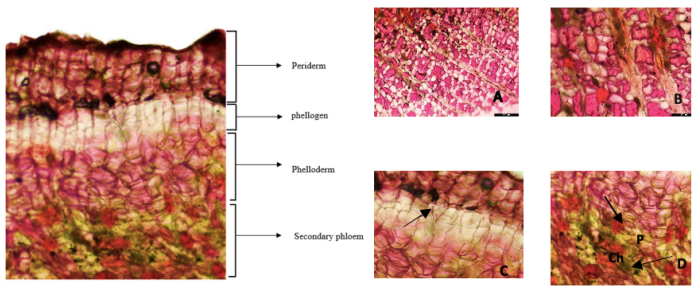
Figure 4: TS of bark *Adina cordifolia* (Roxb.) Brandis



A-Cork cells with content; B-Medullary rays; C-Cells with red pigment; D-Pigmented and granular inclusions; E-Cells with yellow and dark pigments; F-Secondary phloem with contents.

TS of *Mitragyna parvifolia* (Roxb.) Korth can be differentiated into an outer bark (periderm) and an inner bark (secondary phloem). The inner bark is distinguished into collapsed (outer) phloem and collapsed (inner) phloem. The periderm consists of a phellogen layer, a thin zone of phellem and a wide zone of phelloderm. Interior to the phelloderm occurs a wide zone of cortex; cortex cells have undergone widespread divisions and dilations making imaginatively oblong and radially dense cortical tissue. In the cortical zone, stone cells and calcium crystals were seen. Bark shows relatively large calcium oxalate crystals and microcrystals, also known as sand crystals primarily found in the phloem rays. (Figure 5)

Figure 5: TS of bark *Mitragyna parvifolia* (Roxb.) Korth



A: Paranchyema cells; B: Medullary rays; C: Cells with content; D: Cells with orange pigment (P) and chlorophyll (Ch)

Physico-chemical analysis

All three samples showed no presence of foreign matter and were slightly acidic in nature (Ph >5). Moisture content varied across samples; recorded at 6.53%, 3.11%, and 1.98% among *Neolamarckia cadamba* (Roxb.)Bosser, *Adina cordifolia* (Roxb.)Brandis, *Mitragyna parvifolia* (Roxb.) Korth respectively. In all cases, the water-soluble extractive values (10.40%, 21.21%, and 12.87%) were higher than the alcohol-soluble extractive values (7.17%, 14.57%, and 10.41%). (Table 1)

Table 1: Physico-Chemical Parameters of Sample 1 (*Neolamarckia cadamba* (Roxb.) Bosser), Sample 2 (*Adina cordifolia* (Roxb.) Brandis), Sample 3 (*Mitragyna parvifolia* (Roxb.) Korth)

Parameter	*Sample 1*	*Sample 2*	*Sample 3*
	Results (%w/w, Mean ± SD (n = 3)		
Moisture content (%)	6.53 ± 0.01	3.11 ± 0.01	1.98 ± 0.01
pH value	4.35 ± 0.01	4.70 ± 0.01	4.78 ± 0.01
Total Ash (%)	2.37 ± 0.08≤9.0*	5.38 ± 0.04	7.62 ± 0.05
Acid InsolubleAsh(%)	0.37 ± 0.01≤ 1.5*	0.39 ± 0.01	0.59 ± 0.02
Water InsolubleAsh(%)	2.50 ± 0.01	2.50 ± 0.02	2.50 ± 0.02
Alcohol soluble extractive (%)	7.17 ± 0.02≥ 3.0*	14.57± 0.01	10.41±0.02
Water soluble extractive (%)	10.40 ± 0.02≥ 5.0*	21.21± 0.01	12.87± 0.01
Foreign Matter (%)	0.00 ± 0.00≤ 2.0*	0.00 ± 0.00	0.00 ± 0.00
*API Limits = Ayurveda Pharmacopeia of India Limits			

Phyto-chemical analysis

Neolamarckia cadamba (Roxb.)Bosser show the presence of alkaloids, reducing sugar, tannin, phenol, flavonoids, proteins and glycosides in all three extracts. Resins were absent in the aqueous extract. Starch, quinones were detected in methanol and aqueous extract (Table 2). Occurrences of alkaloids, steroids, carbohydrates, reducing sugar, tannin, phenol and glycoside were found in all four extracts of *Adina cordifolia* (Roxb.)Brandis. Coumarins were recorded in aqueous extract and starch was absent in chloroform extract and resin in acetone (Table 3). *Mitragyna parvifolia* (Roxb.)Korth exhibited reducing sugar, tannins and phenols in all four extracts. Steroid was not found in chloroform whereas starch was absent in all four extracts. Quinones were present only in acetone and resin in the aqueous extract. Terpenoids were absent in chloroform extract (Table 4). Certain phytochemical classes showed qualitative reactions in solvents beyond their commonly expected solvent systems. This may be attributed to differences in constituent polarity, sugar-bound, free-base, matrix interaction, conjugation, condensation, and possible anabolic/catabolic interconversion or co-extraction of phytoconstituents during extraction. Therefore, the observed reactions are interpreted as a preliminary qualitative crude-extract response rather than the absolute solvent-specific solubility of individual phytoconstituents. (18,19)

Table 2: Phyto-chemical constituents of *Neolamarckia cadamba* (Roxb.) Bosser

Test	Methanol	Acetone	Chlorofor	Aqueous
Alkaloids	+	+	+	+
Steroid	+	+	-	+
Carbohydrate	+	+	-	+
Reducing	+	+	+	+
Tannin	+	+	+	+
Phenol	+	+	+	+
Flavanoids	+	+	+	+

Proteins	+	+	+	+
Terpenoids	+	-	+	_
Glycoside	+	+	+	+
Resins	+	+	+	-
Starch	+	-	-	+
Quinones	+	-	-	+
Caumarins	-	-	-	-

Table 3: Phytochemical of *Adina cordifolia* (Roxb.) Brandis

Test	Methanol	Acetone	Chlorofor	Aqueous
Alkaloids	+	+	+	+
Steroid	+	+	+	+
Carbohydrate	+	+	+	+
Reducing	+	+	+	+
Tannin	+	+	+	+
Phenol	+	+	+	+
Flavanoids	+	+	-	+
Proteins	+	+	-	+
Terpenoids	+	+	-	+
Glycoside	+	+	+	+
Resins	+	-	+	+
Starch	+	+	-	+
Quinones	+	+	-	-
Caumarins	-	-	-	+

Table 4: Phyto-chemical constituents of *Mitragyna parvifolia* (Roxb) Korth

Test	Methanol	Acetone	Chlorofor	Aqueous
Alkaloids	+	+	+	+
Steroid	+	+	-	+
Carbohydrate	-	-	-	+
Reducing	+	+	+	+
Tannin	+	+	+	+
Phenol	+	+	+	+
Flavonoids	+	-	-	+
Proteins	+	+	-	-
Terpenoids	+	+	-	+
Glycoside	-	-	+	-
Resins	-	-	-	+
Starch	-	-	-	-
Quinones	-	+	-	-
Coumarins	-	-	-	-

High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC profiling of the ethanolic bark extracts revealed distinct chromatographic banding patterns under UV 254 nm, UV 366 nm, and post-derivatization conditions, indicating interspecies variation among the studied samples. *Neolamarckia cadamba* (Roxb.) Bosser showed 8 peaks with a total peak area of 2665.3 AU at 254 nm and 9 peaks with a total peak area of 24023.8 AU at 366 nm, indicating a comparatively greater band distribution. *Adina cordifolia* (Roxb.) Brandis exhibited 3 peaks with a total peak area of 32009.1 AU at 254 nm and 8 peaks with a total peak area of 31943.3 AU at 366 nm, with distinct, well-resolved chromatographic zones. *Mitragyna parvifolia* (Roxb.) Korth showed 2 peaks with a total peak area of 1204.1 AU at 254 nm and 8 peaks with a total peak area of 17725.6 AU at 366 nm, indicating fewer but distinct chromatographic bands. Variations in Rf values, fluorescence intensity, band resolution, and peak area distribution may reflect differences in the polarity, concentration, and composition of extractable phytoconstituents among the bark extracts studied. Thus, the HPTLC profiles obtained at different wavelengths and under different derivatization conditions may serve as supporting chromatographic fingerprints for comparative identification and standardization of the studied species. (Figure 6) (Table 5&6)

Table 5: HPTLC profile Sample 1 (*Neolamarckia cadamba* (Roxb.)Bosser), Sample 2 (*Adina cordifolia* (Roxb.) Brandis), Sample 3 (*Mitragyna parvifolia* (Roxb.) Korth) at UV 254 nm

Species	No.of Peaks	Major Rf Values	Highest % area	Interpretation
Sample 1	9	0.01, 0.19, 0.24, 0.34, 0.37, 0.42, 0.54, 0.75, 0.88	36.78% at Rf 0.75	Comparatively complex profile with a major band at a higher Rf
Sample 2	8	0.14, 0.20, 0.29, 0.32, 0.37, 0.43, 0.53, 0.76	32.72% at Rf 0.43	Moderate profile with dominant mid-Rf peak
Sample 3	8	0.04, 0.12, 0.33, 0.37, 0.42, 0.54, 0.62, 0.77	41.72% at Rf 0.77	Characteristic profile with a dominant higher-Rf peak

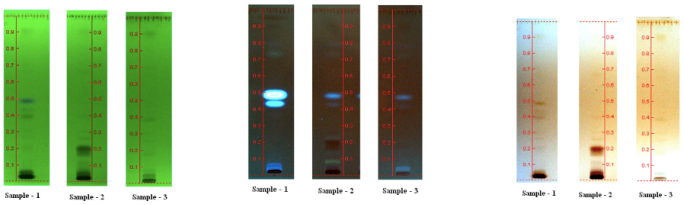
Rf = retention factor; AU =area unit. The major peak was chosen according to the highest percentage area.

Table 6: HPTLC profile of Sample 1(*Neolamarckia cadamba* (Roxb.) Bosser), Sample 2 (*Adina cordifolia* (Roxb.) Brandis), Sample 3 (*Mitragyna parvifolia* (Roxb.) Korth) at UV 366 nm

Species	No.of peaks	Major Rf values	Highest % area	Interpretation
Sample 1	5	0.01, 0.06, 0.38, 0.43, 0.70	40.54% at Rf 0.70	Distinct fluorescent band distribution
Sample 2	3	0.37, 0.43, 0.70	60.26% at Rf 0.37	Prominent fluorescent mid-Rf band
Sample 3	2	0.15, 0.37	56.70% at Rf 0.37	Limited but distinct fluorescent bands

Rf = retention factor; AU =area unit. The major peak was chosen according to the highest percentage area.

Figure 6: Photo documentation of HPTLC view of Sample 1 (*Neolamarckia cadamba* (Roxb.) Bosser), Sample 2 (*Adina cordifolia* (Roxb.)Brandis), Sample 3 (*Mitragyna parvifolia* (Roxb.) Korth)



Discussion

Bark samples exhibited notable macroscopic differences useful for identification and quality assessment. *Neolamarckia cadamba* (Roxb.)Bosser was dark brown with vertically grooved, rectangular exfoliation and a yellowish blaze that darkened over time, suggesting oxidative changes. *Adina cordifolia* (Roxb.)Brandis showed greyish-black bark with small patchy exfoliation and a reddish-pink, waxy blaze, possibly indicating resinous content. *Mitragyna parvifolia* (Roxb.)Korth had grey bark with thin, scaly exfoliation and a pink blaze that turned red on exposure, suggesting chemical transformation.

Microscopic evaluation of the three samples revealed distinct cellular composition. *Neolamarckia cadamba* (Roxb.)Bosser predominantly contained pigment cells along with starch and crystal cells indicating a moderate presence of storage and mineral content. *Adina cordifolia* (Roxb.)Brandis exhibited an abundance of pigment cells, particularly red and orange along with calcium crystals and starch cells suggesting higher metabolic activity. *Mitragya parvifolia* (Roxb.)Korth in contrast showed a dominance of chlorophyll containing cells, orange pigment cells, and crystal cells, reflecting a possible photosynthetic contribution along with mineral deposition. The variation in pigment types and crystal abundance across samples may be indicative of differences in geographical area, stages of maturity, environmental conditions influencing phytochemical composition (20).

Physiochemical findings suggest that all three samples were free from foreign matter and exhibit slightly acidic pH, indicating a consistent acidic nature across the test drugs. Notably the water-soluble extractive values were higher than the alcohol soluble ones in all samples, highlighting the predominance of water-soluble phytoconstituents suggesting better for pharmaceutical preparations like decoction.

Phytochemical screening revealed common and unique characteristics across the three samples. All extracts from the samples contained alkaloids, reducing sugars, tannins, phenols and glycosides suggesting the presence of shared bioactive compounds. *Neolamarckia cadamba* (Roxb.)Bosser was distinguished by the presence of flavonoids, proteins, terpenoids, with no resins in the aqueous extract, indicating a more complex chemical composition in polar solvents. *Adina cordifolia* (Roxb.)Brandis contained steroids, coumarins, and quinones, but lacked starch in chloroform and resins in acetone, suggesting a different solubility and extraction profile. *Mitragya parvifolia* (Roxb.)Korth however was unique in its complete lack of starch and absence of steroids, terpenoids in chloroform. These differences indicate solvent-specific solubility, polarity, matrix interaction, conjugation, condensation, and possible anabolic/ catabolic interconversion or co-extraction of phytoconstituents during extraction. (18) (21)

HPTLC profiling of the ethanolic extract of three drugs exhibited distinct chromatographic banding patterns when observed under varying wavelengths of light, indicating the presence of diverse phytoconstituents. Comparing the HPTLC profiles, *Neolamarckia cadamba* (Roxb.)Bosser displayed more peaks(8) with significant areas at both 254 nm and 366 nm, with the highest peak at 366 nm (24023.8 AU), while *Adina cordifolia* (Roxb.)Brandis showed fewer peaks (3) but higher areas at both wavelengths, particularly at 254 nm (32009.1 AU). *Mitragya parvifolia* (Roxb.)Korth on the other hand, exhibited the fewest peaks (2) and lower area percentages, particularly at 254 nm, suggesting a less complex phytochemical profile compared to others.

Conclusion

The pharmacognosy study on the bark of *Neolamarckia cadamba* (Roxb.)Bosser, *Adina cordifolia* (Roxb.)Brandis and *Mitragya parvifolia* (Roxb.)Korth aligns with the primary goal of establishing the correct identity and quality standards for these herbs, providing a foundation for their safe and effective use in traditional medicine. Findings provide unique signatures of each sample's constituents, showing distinct phytochemical profiles for all three. The three samples share common bioactive compounds such as alkaloids, tannins and glycosides as well as slightly acidic pH and higher water-soluble extractive values, making them suitable for similar medicinal purposes. They differ in their macroscopic features, microscopic compositions, and specific phytochemical profiles. The comprehensive profiling of all three samples serves as a critical basis for further research, quality control tool in medicinal applications.

Conflict of Interest: Nil

Financial Support: Nil

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