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Comparative Phytochemical and FTIR Evaluation of *Morinda lucida* Leaves from Two Locations within the University of Ibadan

Segun A. Aderibigbe[1], Oluwatobi O. Olakojo[2]* and Latifat O. Fasasi[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria*
[2]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Federal University, Oye-Ekiti, Ekiti, Nigeria*

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ABSTRACT

Morinda lucida is widely used in African traditional medicine; however, variability in its phytochemical composition may affect its medicinal quality. This study aimed to comparatively evaluate the phytochemical profile and Fourier-transform infrared (FTIR) characteristics of *M. lucida* leaves obtained from two locations within the University of Ibadan: Animal House, near the Faculty of Basic Medical Sciences, and Teaching and Research Farm. The solvent extracts, obtained by macerating pulverized dried leaves with chloroform and acetone, were evaluated using phytochemical screening, thin layer chromatography, and infrared spectroscopy. Comparisons were made between the datasets. Phytochemical screening revealed more variations in the phytochemical composition of the acetone extracts of both samples compared with the chloroform extracts. The infrared spectra of chloroform extracts appeared to match closely; however, noticeable variations in the spectra of acetone extracts. The results indicated possible variations in the phytochemical composition of *M. lucida* (leaves) collected from the same geographical locations. This emphasizes the need for enhanced standardization protocols and quality assurance of *M. lucida* morphological parts used for herbal formulations, even within the same geographic area.

Keywords: *Morinda lucida*, Infrared spectroscopy, Phytochemical analysis, Thin layer chromatography.

Introduction

In African traditional medicine, medicinal plants are used extensively in the treatment of diseases.[1] These plants are rich in secondary metabolites (SMs), some of which are the bioactive constituents with several pharmacological roles, including anti-inflammatory, antioxidant, antibacterial, antimalarial, and anticancer activities.[2,3] It is well established that many of the available pharmaceuticals and medicinal agents being used in modern therapy are from plants.[2,4] Recent ethnobotanical reviews have emphasized the continued therapeutic relevance of indigenous African plants and the need for detailed phytochemical validation of traditionally used species.[5]

Morinda lucida Benth (Family: Rubiaceae), commonly known as brimstone tree, is an evergreen medium-sized tree. It is used in traditional medicine in the management of diabetes, hypertension, malaria, and problems with the digestive system. It is widely grown across West and Central Africa, and is known by various local names in these regions, including Oruwo (Yoruba), Nfia/Eze Ogu (Igbo), Njisi (Hausa), Kpan ligom (Tiv), Ugigo (Ebira), all in Nigeria.[6] While different morphological parts of the plant are used medicinally in the treatment of diseases, the leaf is the most sustainable compared with the root or stem bark. The leaf is traditionally used as a medicinal tonic, a remedy against fever, a decoction for malaria treatment, in the management of sickle cell disease, hypertension, and rheumatic disorders, as well as an antidiabetic remedy.[6]

*Corresponding author. Email: oluwatobi.olakojo@fuoye.edu.ng
Tel.: 08059774566

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Many of these uses have been validated scientifically.[7] In contrast to the plant's ethnobotanical relevance, the phytochemical composition of *Morinda lucida* has drawn scientific attention due to its diverse array of SMs. Previous phytochemical studies[6,7] have reported the presence of anthraquinones, monoterpenes, sesquiterpenes, and fatty acids, which are considered chemotaxonomic markers of the species. In addition, other compounds such as alkaloids, tannins, and flavonoids have been identified, contributing to the pharmacological basis of the plant's traditional uses. The distribution of SM compositions and quantities in medicinal plants varies depending on a number of factors, including environmental and agronomical factors.[8-11] Similar concerns regarding cultivation and environmental influences on medicinal plant quality have been reported among smallholder farmers in South Africa.[12] While variation in the SMs composition in plants is well documented as it occurs among different morphological parts as well as across circadian and annual cycles, it is often taken for granted that similar morphological parts of the same plants collected in the same season and within the same geographical area should contain relatively similar SMs qualitatively if not quantitatively. Variability of medicinal plant materials with regard to secondary metabolite composition can alter the therapeutic performance expected of plant-based medicinal products.[13,14] In this context, thin-layer chromatography (TLC) and infrared (IR) spectroscopy serve as important analytical tools for the rapid profiling and fingerprinting of plant extracts.[14,15] These techniques help detect qualitative and semi-quantitative differences in SMs and provide functional group-level identification, thereby supporting the authentication and standardization of plant materials used in traditional and modern phytomedicine.

The study addresses phytochemical variability of *M. lucida* leaves collected from two sites within the same geographical area, an aspect that remains underreported. The aim of this research was therefore to comparatively investigate the phytochemical composition and FTIR spectral characteristics of *M. lucida* leaves from these two locations, with a view to establishing intra-species variability and implications for standardization of herbal preparations.

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Materials and Methods

Chemicals and reagents

Reagents used were all of analytical grade. These include Acetone (≥99.5%, Abcr, Germany), chloroform (≥99.8%, JHD, China), sulphuric acid (95–98%, BDH Chemicals, UK), ferric chloride (≥97%, BDH Chemicals, UK), ammonia solution (25%, Merck, Germany), acetic acid (≥99.7%, Fisher Scientific, USA), n-hexane (≥99%, Merck, Germany), ethanol (≥99.8%, JHD, China), ethyl acetate (≥99.5%, Merck, Germany), and methanol (≥99.9%, JHD, China).

Plant collection and identification

Morinda lucida leaves were collected in Oct. 2021, from two different locations, both within the University of Ibadan: Teaching and Research Farm (TRF; 7.4560697°N, 3.8939033°E), and around the Animal House (AH), Faculty of Basic Medical Sciences (7.449113°N, 3.905452°E). The two locations were essentially similar in terms of humidity, rainfall, and sunlight. Voucher specimens were deposited at the Forestry Research Institute of Nigeria, Jericho, Ibadan, for authentication (TRF sample: 1142074; AH sample: 113370). Distilled water was used to rinse the collected samples to remove dust and dirt. The leaves were air-dried at room temperature for over 14 days, after which the dried material was pulverized into coarse powder using an industrial blender.

Plant extraction

The powder materials (30 g) were macerated in 100 mL of *n*-hexane for 24 h to remove lipids and pigments. This pretreatment step helps to eliminate non-target constituents that may interfere with subsequent analyses. The air-dried defatted materials were then macerated in fresh chloroform (100 mL for 24 h, three times), and this was successively followed by macerating in fresh acetone (100 mL, three times). Chloroform was selected as a non-polar solvent to preferentially extract lipophilic compounds such as sterols, terpenoids, and certain anthraquinones, while acetone, being a polar solvent, was chosen to target more polar constituents such as flavonoids, tannins, and glycosides. The supernatants were pooled and concentrated on a rotary evaporator at 40°C to smaller volumes. The concentrates were dried *in vacuo* for 48 h at 40°C. The dried extracts were weighed and stored in labelled glass vials.

Phytochemical evaluation of extracts

Phytochemical evaluations were carried out on both the chloroform and acetone extracts of the samples using standard methods as described by Evans[16] and Ayoola and co-workers.[17]

Thin layer chromatographic evaluation of extracts

Aliquots (about 2 mg) of each extract, dissolved in 2 mL of chloroform or acetone as appropriate, were spotted on silica gel plates (F_{254}, aluminum sheets 10×5 cm, Merck, Germany). The spotted plates were allowed to dry and then developed in different solvent systems to achieve optimal separation. Chloroform extracts were developed in ethyl acetate/ *n*-hexane (30/70), and xylene/ethyl acetate/triethylamine (70/30/5), while acetone extracts were developed in ethyl acetate/methanol (30/70), and ethyl acetate/ethanol/acetic acid (90/10/5). After development, the air-dried plates were examined under UV light at 254 and 365 nm.

Infrared spectroscopic evaluation of extracts

The extracts were subjected to infrared spectroscopic evaluation using a Fourier-transform infrared spectrophotometer (Spectrum II, PerkinElmer, USA) equipped with an attenuated total reflectance (ATR) accessory. About 1 mg of each extract was smeared onto the diamond crystal, and spectra were recorded in the 4000–400 cm^{-1} range at a resolution of 4 cm^{-1}.

Results and Discussion

The extraction yield and the result of the phytochemical evaluation of the *M. lucida* leaf extracts from the two samples are presented in Table 1. The phytochemical evaluation revealed the presence of important secondary metabolites in both the chloroform and acetone extracts from the two locations, with slight variations. The chloroform extracts from both sites were largely comparable, showing little variation in most phytochemical classes. In contrast, the acetone extracts revealed marked differences: TRF-A contained stronger saponin and terpenoid signals than AH-A, while anthraquinones were present in AH-A but absent in TRF-A. These differences indicate that polar metabolites of *M. lucida* are more susceptible to environmental or micro-ecological influences than non-polar metabolites, a finding of significance for quality control.

Table 1: Phytochemical evaluation results for *M. lucida* extracts from the two locations

	Plant extracts (Yield, % w/w)			
TESTS	**AH-C (4.8)**	**TRF-C (4.2)**	**AH-A (4.1)**	**TRF-A (3.5)**
Saponins	-	-	+	+
Tannins	-	-	+	+
Flavonoids	-	-	+	+
Cardiac Glycosides	+	+	+	+
Anthraquinones	-	+	+	-
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Alkaloids	-	-	+	+

AH-C: Chloroform extract of *M. lucida* collected at Animal House
TRF-C: Chloroform extract of *M. lucida* collected at Teaching and Research Farm
AH-A: Acetone extract of *M. lucida* collected at Animal House
TRF-A: Acetone extract of *M. lucida* collected at Teaching and Research Farm

Thin-layer chromatography is a valuable technique that is very useful in the analysis and fingerprinting of plants for their secondary metabolites' composition.[18,19] The various chromatographic profiles of the chloroform and acetone extract of the *M. lucida* samples are presented in Figure 1. The profiles provided a detailed overview of the different secondary metabolites present in the samples. For the chloroform extracts of the samples from the two locations, no noticeable difference was observed with EtOAc/n-Hex (30/70) as mobile phase (IA & IB). On the other hand, the same samples with EtOAc/Xylene/ Triethylamine (30/70/5) as mobile phase showed a noticeable difference (IIA & IIB, Figure 1). With regard to the acetone extracts of the samples, the profiles suggested no difference between AH-A and TRF-A, judging by the profiles obtained for IIIA and IIIB, as well as IVA and IVB (different mobile phases and viewing at 254 and 365 nm). The identification of functional groups/structural units using infrared spectroscopy plays an important role for analyzing the possible phytochemical compounds present in plant extracts.[20] Infrared spectroscopy detects the stretching and bending of bonds within functional groups, and the spectrum measured for an extract is basically a sum of the spectra of the individual constituent compounds.[21] The measured spectrum could thus serve as a fingerprint profile of the extract. More importantly, by looking at frequencies above 1400 cm^{-1}, deductions can be made of possible functional groups or chemical bonds present as a result of the fundamental vibrational frequencies observed in this region.[22] The resulting spectra obtained from the infrared measurements of the *Morinda lucida* leaves from both locations are shown in Figures 2 and 3. The absorption frequency data are presented in Tables 2 and 3.

The infrared spectra of the chloroform extracts, AH-C and TRF-C, revealed many fundamental vibrations (Figure 2). The observed vibrations in both include: the medium vibration (3418 cm^{-1}) of the OH group, the intense vibration with splitting for the CH aliphatic group (2850-3000 cm^{-1}), the weak but distinct aldehyde stretching (2726 cm^{-1}), the medium vibration for the carbonyl group (split, 1690-1740 cm^{-}

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[1]), and the medium vibrations of C-O (1376, 1454 cm^{-1}). Overall, the profiles of the two spectra revealed they were relatively superimposable on each other and thus, were in essence similar.

Table 2: Characteristic infrared absorption frequencies and the corresponding functional groups in *Morinda lucida* chloroform extracts from two locations within the University of Ibadan

S/no	*Freq. (cm^{-1})		Possible chemical bond(s)**
	AH-C	TRF-C	
1	3418.44	3418.49	OH (alcohol), SV, Broad
2	2958.97	2960.2	C-H, SV - aliphatic
3	2919.41	2851.84	C-H, SV - aliphatic split
4	2850.47	2923.46	C-H, SV - aliphatic
5	2727.04	2726.42	C-H, WV - aldehyde
6	1738.46	1736.78	C=O, SV - ester, aldehyde
7	1712.57	1712.18	C=O, SV -
8	1695.42	1693.9	C=O, SV – conjugated
9	1625.3	1618.5	C=C
10	1553.4	1554.19	-
11	1515.7	1515.7	-
12	1495.1	1495.1	-
13	1454.77	1453.71	CH_2, BV
14	1376.30	1376.15	CH_3, BV
15	1221	1240.15	C-O
16	1166.33	1161.31	C-O (ester) SV
17	1128.5	1128.5	C-O (tertiary alcohol) SV
18	1088.97	1089.57	C-O (secondary alcohol) SV
19	1035.97	1036.72	C-O
20	981.13	977.7	-
21	919.45	919.45	-
22	895.46	890.75	C=C alkene BV
23	835.27	836.37	C=C (alkene) bending
24	727.56	729.90	C=C (alkene) bending
25	666.61	666.57	-
26	573.06	572.36	-

*Frequency **Reference[19]
AH-C: Chloroform extract of *Morinda lucida* collected at Animal House
TRF-C: Chloroform extract of *Morinda lucida* collected at Teaching and Research Farm
SV: Stretching vibration; WV: Weak vibration; BV: Bending vibration

These patterns were as expected since both extracts were chloroform extracts of the same plant, though of different locations within the university. The intense vibration observed between 2850-3000 cm^{-1} also showed that the chloroform extract contains a lot of CH aliphatic bonds, which was consistent with the presence of large numbers of non-polar compounds in the chloroform extracts. This also conforms to compounds that have been previously isolated from the plant, which include phytosterols, terpenes, terpenoids, and some anthraquinones.[7] Similarly, the weak OH stretching, unlike the prominent and intense CH stretching, also indicated that few amounts of OH-containing compounds are present.

Table 3: Characteristic infrared absorption frequencies and their corresponding functional groups in *Morinda lucida* acetone extracts from two locations within the University of Ibadan

S/no	Freq. (cm^{-1}) AH-A	Possible chemical bonds	*Freq. (cm^{-1}) TRF-A	Possible chemical bonds**
1	3850.06	-	-	
2	3746.18	-	-	
3	3384.18	OH (alcohol), SV, broad	3384.43	OH (alcohol), SV, broad
4	2925.52	C-H, MV - aliphatic	2924.07	C-H, SV - aliphatic, sharp, split
5	2886.3	C-H, MV - aliphatic, shoulder	2855.5	C-H, SV – aliphatic, sharp, split
6	1740.45	C=O, SV - ester, aldehyde	1739.34	C=O. SV, sharp split
7	1697.3	C=O	1686.42	C=O, SV, sharp, split
8	1642.5	C=C, MV	1642.5	C=C. MV
9	1515.73	-	1516.7	-
10	1454	CH_2,	1453.83	CH_2, sharp, MV
11	1382.06	CH_3	1375.43	CH_3, sharp, MV
12	1282.7	-	-	
13	1187.20	C-O, sharp, MV	1186.62	
14	1073.6	C-O,	1073.6	
15	1032.28	C-O, sharp, SV	1031.86	C-O, sharp, SV
16	974.28		974.28	
17	922.88		919.45	
18	864.62		868.05	

*Frequency **Reference[19]
AH-A: Acetone extract of *Morinda lucida* collected at Animal House
TRF-A: Acetone extract of *Morinda lucida* collected at Teaching and Research Farm
SV: Stretching vibrations; MV: Medium vibration

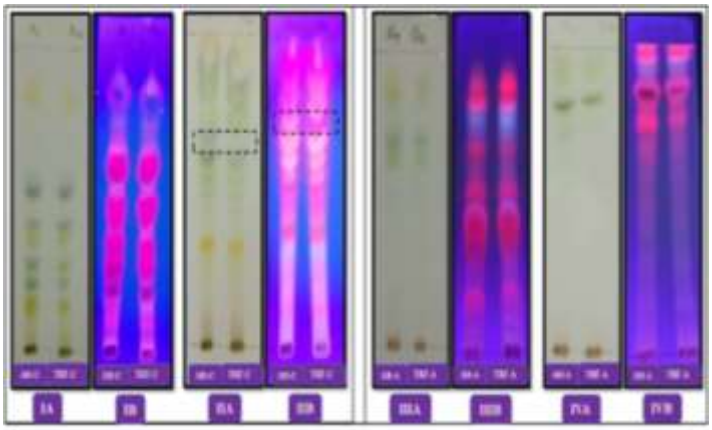

Figure 1: TLC profiles of *Morinda lucida* (leaf) solvents extracts: Chloroform extracts of samples collected at Animal House (AH-C) and Teaching and Research Farm (TRF-C) - IA & IB - viewed at 254 and 365 nm, respectively with EtOAc/*n*-Hex (30/70) as mobile phase, IIA & IIB - viewed at 254 and 365 nm, respectively with EtOAc/Xylene/Triethylamine (30/70/5) as mobile phase; and Acetone extracts of samples collected at Animal House (AH-A) and Teaching and Research Farm (TRF-A) - IIIA & IIIB - viewed at 254 and 365 nm, respectively with EtOAc/MeOH (30/70) as mobile phase; and IVA & IVB - viewed at 254 and 365 nm with EtOAc/EtOH/CH_3COOH (90/10/5) as mobile phase.

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Figure 3 includes the FTIR spectra of the acetone extracts: AH-A and TRF-A. In contrast to Figure 1, the overall profiles of the two spectra revealed that they were not superimposable on each other, nor was there a perfect match. A comparative summary of the key infrared absorption bands observed across the samples, their assigned functional groups, and the corresponding phytochemical classes is presented in Table 4. This provides further evidence supporting the phytochemical screening data and highlights subtle chemical differences between the extracts from the two locations. The observed fundamental vibration disparities between the two include: the medium CH vibration (2925 cm^{-1}) with a shoulder at 2886 cm^{-1} (AH-A) compared with intense CH stretching vibration (2924 cm^{-1}) along with a sharp peak at 2855 (TRF-A); and the strong carbonyl vibration stretching at 1740, 1697 cm^{-1} (AH-A) compared with a similar strong carbonyl vibration stretching but with a split at 1739 and 1686 cm^{-1} (TRF-A). The few similarities include the intense OH stretching at 3384 and the C-O stretching vibration (1031, 1032 cm^{-1}).

Whereas the chloroform extracts (AH-C and TRF-C) produced nearly the same FTIR spectra, confirming compositional similarity, the acetone extracts (AH-A and TRF-A) displayed clear divergence in the CH and carbonyl stretching regions. These spectral variations corroborate the phytochemical screening results and highlight specific functional groups that may account for observed differences in phytoconstituents between the two sites.

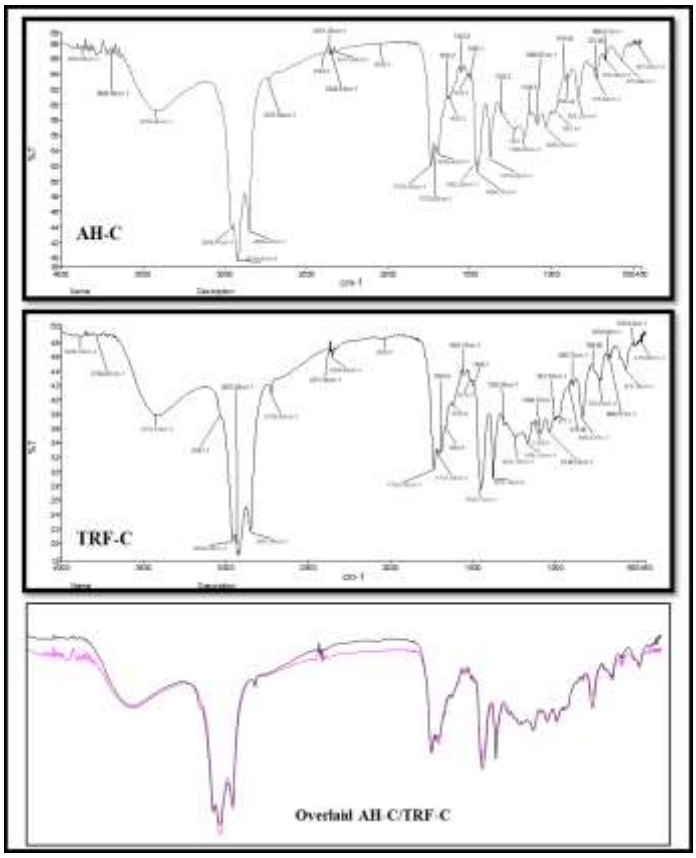

Figure 2: Infrared spectra of chloroform extracts of *M. lucida* collected at Animal House (AH-C) and Teaching and Research Farm (TRF-C), and their overlaid spectra

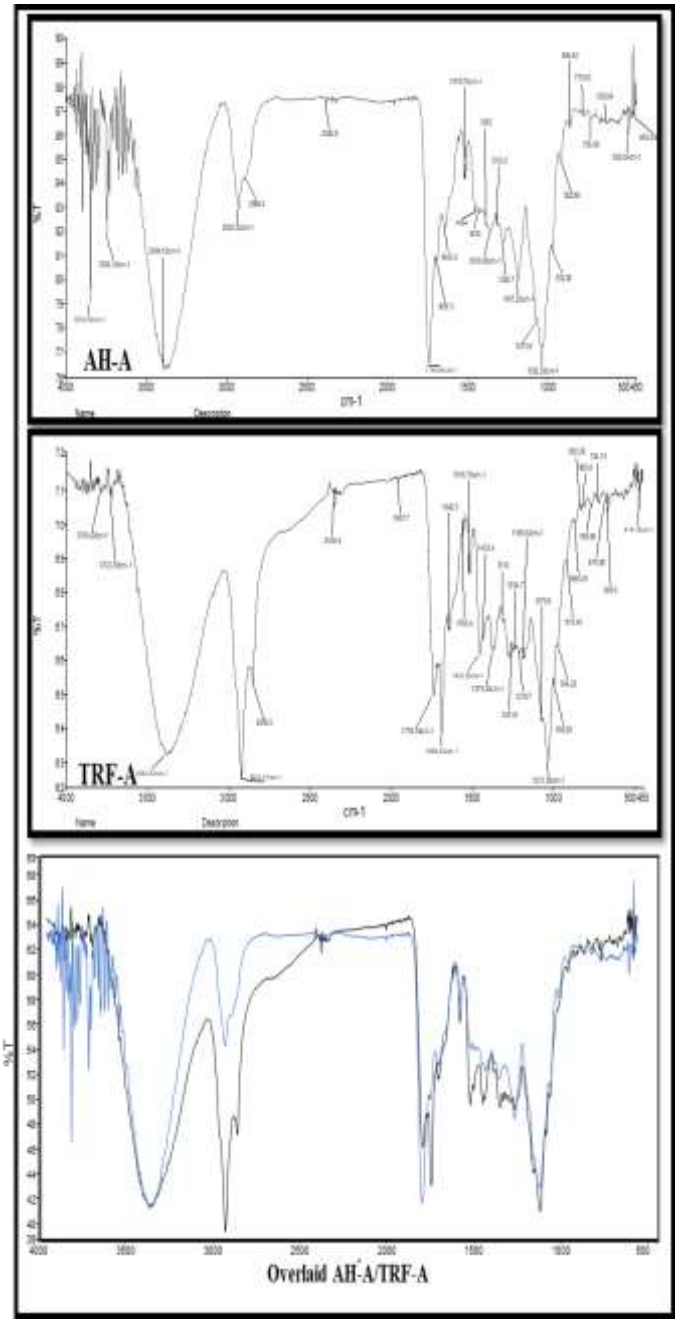

Figure 3: Infrared spectra of acetone extracts of *M. lucida* collected at Animal House (AH-A) and Teaching and Research Farm (TRF-A), and their overlaid spectra

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Table 4: Comparative summary of IR absorption bands, functional groups, and corresponding phytochemical classes in *Morinda lucida* leaf extracts

Functional group/ Band	Frequency range (cm^{-1})	AH-C	TRF-C	AH-A	TRF-A	Likely phyto-chemical class	Remarks
O–H (broad stretch)	~3384–3418	✓	✓	✓	✓	Flavonoids, tannins, saponins	Present in all, more prominent in acetone extracts
C–H (aliphatic stretch)	~2850–2960	✓	✓	✓ (2925, 2886)	✓ (2924, 2855)	Terpenoids, sterols, fatty acids	Similar pattern; shoulder in AH-A, sharp in TRF-A
C=O (carbonyl stretch)	~1690–1740	✓ (split)	✓ (split)	✓ (1740, 1697)	✓ (1739, 1686)	Anthraquinone, glycosides	Present in all; more splitting in TRF-A
C=C (alkene stretch)	~1625–1642	✓	✓	✓	✓	Phenolics, anthraquinones, flavonoids	Present and comparable in all samples
C–O (alcohol/ ester)	~1031–1240	✓	✓	✓	✓	Flavonoids, tannins, glycosides	Consistent across all extracts
Aldehyde C–H (weak)	~2726	✓	✓	-	-	Flavonoids /Triterpenes with aldehydes	Observed only in chloroform extracts
CH_2/CH_3 bending	~1376–1454	✓	✓	✓	✓	Lipids, terpenoids	Similar presence in all
Minor peaks	~800–1300	✓	✓	✓	✓		Consistent, not diagnostic
Overall similarity		High	High	Moderate	Moderate		Chloroform: highly similar; Acetone: more variability

Note: AH = Animal House, University of Ibadan; TRF = Teaching and Research Farm, University of Ibadan. AH-C/TRF-C = chloroform extracts; AH-A/TRF-A = acetone extracts. ✓ = band present; "split" = band splitting observed.

Conclusion

This study shows that significant phytochemical variability can exist even within the same species collected from nearby sites, underscoring the need for rigorous standardization. Across the levels of investigation conducted, variations in phytochemical compositions were observed. These demonstrate the importance of applying multiple analytical approaches to ensure reliable standardization and quality control of herbal materials. This is of considerable importance for quality standard and minimizing batch-to-batch variation of plant-based drugs.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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