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Research Paper

Rapid Screening of Adulterants of *Mesua ferrea* L. by FTIR and GC-MS Techniques

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ABSTRACT

Nagkesar, derived from the stamens of *Mesua ferrea* L., is frequently substituted with unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb. both knowingly and unknowingly in the Indian markets. The goal is to establish a reliable method for distinguishing between the genuine plant and its substitutes by leveraging the unique spectral characteristics revealed by FTIR and GC-MS analyses. Powdered plant samples underwent Fourier transform infrared (FTIR) spectrum analysis using the Bruker, Germany Vertex 80 FTIR System with a 3000 Hyperion Microscope. The samples were scanned from 4000 to 440 cm^{-1} , and the recorded peak values of the plant materials were documented. Additionally, Gas chromatography–mass spectrometry (GC-MS) analysis was conducted on the soxhleted methanolic extract of stamens of *Mesua ferrea* L., unripe fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb...Chromatograms obtained were studied, and compound identification was accomplished by comparing the spectrum of unknown compounds with that of known compounds. The Fourier transform infrared (FTIR) spectrum confirmed the presence of various functional groups, including alcohols, phenols, alkanes, alkenes, carbonyls, alkyl halides, and aromatic compounds in all plant samples, each with peaks at distinct absorptions. FTIR analysis revealed unique spectrum patterns facilitating the differentiation of the adulterants. Gas chromatography–mass spectrometry analysis identified the presence of specific compounds such as 2-Propanone, 1- hydroxy ($\text{C}_3\text{H}_6\text{O}_2$), Hexanoic acid, 2- methyl ($\text{C}_7\text{H}_{14}\text{O}_2$), 1b, 4a- Epoxy-2H-cyclopenta[3,4] cyclopropa[8,9]cycloundec[1,2-b]oxiren-5 (1aH0-one, 2,7,9,19- tetrakis (acetyloxy) decahydro-3,6,8,10a-pentamethyl ($\text{C}_{28}\text{H}_{38}\text{O}_{11}$), Furanacetic acid, 4- hexyl-2,5-dihydro-2,5-dioxo ($\text{C}_{12}\text{H}_{16}\text{O}_5$), Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester ($\text{C}_{16}\text{H}_{26}\text{O}_2$)

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in the stamens of *Mesua ferrea* L., serving as potential indicators for detecting adulteration. The spectral fingerprints established a clear distinction between the genuine *Mesua ferrea* L. and its potential adulterants. The variations observed in the FTIR and GC-MS chromatograms provide robust criteria for identifying and confirming the presence of adulteration in Nagkesar samples.

INTRODUCTION

Medicinal plants have long been utilized for their unique phytoconstituents, offering therapeutic benefits and promoting health[1]. *Mesua*, a substantial genus encompassing approximately 48 species, with *Mesua ferrea* L. being the most extensively studied, stands out as an epitome. This species is endangered in India, native to tropical Sri Lanka, and holds the official status of being the tree of Tripura. Commonly known as Cobra's saffron in English and *Nagakeshara* in Hindi, it continues to be a subject of significant exploration and interest [2]. In Ayurveda, 'Nagkesar' holds a prominent position as a crucial medicinal remedy, with *Mesua ferrea* L.'s stamen being its authentic source. The therapeutic effects of *M. ferrea* L. stamens are evident by the presence of steroids, terpenoids, and volatile oil components. These stamens demonstrate notable bactericidal and antioxidant activities [3]. Despite these benefits, market samples often suffer from adulteration, with unripe fruits of *Cinnamomum tamala* T.Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb. being falsely presented as 'Kala nagkesar' and 'Malabar nagkesar' respectively. 'Kala nagkesar' are sold in the markets of Gujrat and Bombay whereas 'Malabar nagkesar' in South India. The substituted products lack any connection to the genuine drug and may or may not contain essential phytoconstituents [4], [5] Thus, the development of biomarkers becomes crucial for distinguishing between authentic and adulterated plant samples. The objective of this present study was to develop FTIR and GC-MS fingerprints of *Mesua ferrea* L., *Cinnamomum*

tamala T. Nees & Eberm. and *Dillenia pentagyna* Roxb. This approach seeks to distinguish authentic *Mesua ferrea* L. from its adulterants, ensuring the integrity and quality of this valuable medicinal plant in the market.

MATERIALS AND METHODS

Collection and authentication of plant materials

The blooms of *Mesua ferrea* L. were gathered in February from Veermata Jijabai Bhosale Udyan in Mumbai. Additionally, the immature fruits of *Cinnamomum tamala* T. Nees & Eberm. and the fruits of *Dillenia pentagyna* Roxb. were acquired in June from a botanical garden in Dahanu (Palghar, Maharashtra), and Dapoli Urban Bank Senior Science College (Jalgaon, Maharashtra) respectively. The procured plant sample *Mesua ferrea* L. was identified and authenticated at Blatter Herbarium, St. Xavier's College, Fort, Mumbai. The voucher specimen number given was NDG-2259.

Preparation of sample powder

The flowers of *Mesua ferrea* L., unripe fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb. were air-dried in the shade for one week. Later, the stamens from *Mesua ferrea* L. flowers were separated and ground into powder using a mixer blender. The shade-dried unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb. were also mechanically ground.

FTIR Spectrum Analysis

To identify the characteristic functional groups present, the powdered plant samples underwent Fourier transform infrared (FTIR) spectrum analysis. This analysis was conducted at the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Mumbai. The FTIR spectra



were generated using the Bruker, Germany Vertex 80 FTIR System with a 3000 Hyperion Microscope. The samples were scanned across the range of 4000 to 440 cm^{-1} , and the recorded peak values of the plant materials were documented.

GC-MS analysis

The GC-MS analysis of the soxhleted methanolic extract derived from the stamens of *Mesua ferrea* L., unripe fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb. was conducted at the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. This analytical process aims to examine the presence of active constituents and the chemical composition of the samples. The equipment utilized for this analysis included the Agilent 7890 GC instrument and the Joel Accu TOF GCV MS instrument. The solvent mixture employed consisted of toluene, chloroform, ethanol, and ethyl acetate. Helium gas (99.999%) served as the carrier gas with a flow rate of 1 ml/min. The analytical setup featured an HP5 column with specifications including a length of 30 mm, internal diameter of 0.32 mm, film thickness of 0.25 mm, and a temperature range from -60 °C to 325 °C (350 °C). The GC runtime was set at 35 minutes, during which the oven temperature increased from 70 °C to 280 °C at a rate of 8 °C per minute. A sample size of 4 μl was injected through the injector. The MS was performed at 70eV. Compound identification was achieved by comparing the spectrum of unknown compounds with the spectrum of known compounds in the library, enabling the determination of their names, molecular weights, and structures.

RESULTS

FTIR spectrum analysis

The powdered stamens of *Mesua ferrea* L. exhibited 11 distinct functional groups, as indicated by the FTIR spectrum. Table 1 and Figure 1 illustrate the FTIR spectrum specific to *M. ferrea* L. stamens. The absorption peak at 3416.73 cm^{-1} corresponds to the stretching vibration of the H-bonded and O-H groups. Additionally, the peaks at 2926.42 and 2854.43 cm^{-1} signify the asymmetric and symmetric stretching of saturated (sp^3) carbon, respectively. The band at 1729.48 cm^{-1} is attributed to C=O stretching related to the carbonyl skeletal mode in the plant sample. Various other groups, including aromatic compounds, phenols or tertiary alcohols, acids, alkenes, alkyl halides, phosphate ions, and halogen compounds, exhibit absorption at 1525.27, 1445.81, 1376.38, 1341.51, 1284.42, 1244.89, 1656.27, 823.48, 779.32, 702.18, 1158.29, 1101.79, 614.29, 559.31, and 518.47 cm^{-1} , respectively. The identification of the functional group in unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. relied on the analysis of the FTIR spectrum, focusing on specific peak values within the infrared radiation range. Figure 2 and Table 2 present the FTIR spectrum for unripe fruits of *Cinnamomum tamala* T. Nees & Eberm... A prominent absorption band at 3433.07 cm^{-1} indicated the presence of bonded H/O-H stretch. The C-H asymmetrical stretching methylene group was observed at 2922.86 cm^{-1} , while the band at 2852.33 cm^{-1} represented the C-H symmetric stretching of methylene groups in aliphatic compounds. The absorbance at 1639.64 cm^{-1} signified the existence of carboxyl compounds (C=O stretch). Other observed bands included 1459.37 cm^{-1} (C=C stretch), 1381.85 cm^{-1} (O-H bend), and 1048.78 cm^{-1} (PO^3 stretch). FTIR spectroscopy was employed to analyze the functional groups present in the powdered fruits of *Dillenia pentagyna* Roxb. (Figure 3 and Table 3), with the separation of these groups based on peak values. An expansive absorption band at 3734.82



and 3430.78 cm^{-1} signifies the presence of non-bonded, O-H stretch and H-bonded, O-H stretch respectively. A narrower band at 2924.6 cm^{-1} indicates asymmetric stretch and at 2853.26 cm^{-1} shows symmetric stretch in saturated aliphatic compounds. A strong intensity band at 1640.88 cm^{-1} reveals a C=O stretch, indicating the presence of a ketone compound. Peaks at 1373.99 , 1321.99 , and 1230.32 cm^{-1} are assigned to the O-H bend and

C-O stretch, respectively. The absorption band at 1108.28 cm^{-1} is attributed to amine and alkyl halide. An intense band at 1051.08 cm^{-1} corresponds to PO_3 stretch vibration, indicating the presence of phosphate ions. Multiple bands at 884.31 , 829.35 , 783.35 cm^{-1} confirm the presence of alkenes. Peaks at 607.03 and 522.90 cm^{-1} are associated with halogen compounds.

Table 1: Structural features of the *M. ferrea* L. stamens by FTIR spectrum

Wave numbers (cm^{-1})	Functional Group	Functional Group Name	Vibrations
3416.73	O-H	Hydroxyl	Stretch, Bonded
2926.42	C-H	Lipids	Asymmetric stretch
2854.43	C-H	Fatty acids, Lipids, Proteins	Symmetric stretch
1729.48	C=O	Carbonyl	Stretch
1656.27	C=C	Alkene	Stretch
1525.27	C=C	Aromatic ring	Stretch
1445.81	C=C	Aromatic ring	Stretch
1376.38	O-H	Phenol or tertiary alcohol	Bend
1341.51	O-H	Phenol or tertiary alcohol	Bend
1284.42	C-O	Acid	Stretch
1244.89	C-O	Acid	Stretch
1158.29	C-N, C-H	Amine, Alkyl halide	Stretch
1101.79	PO_3	Phosphate ion	Stretch
823.48	=C-H	Alkene	Bend
779.32	=C-H	Alkene	Bend
702.18	=C-H	Alkene	Bend
702.18	C-I, C-Cl	Alkyl halide	-
614.29	C-I, C-Cl	Alkyl halide	-
559.31	C-I, C-Cl	Alkyl halide	-
518.47	C-I, C-Cl	Alkyl halide	-

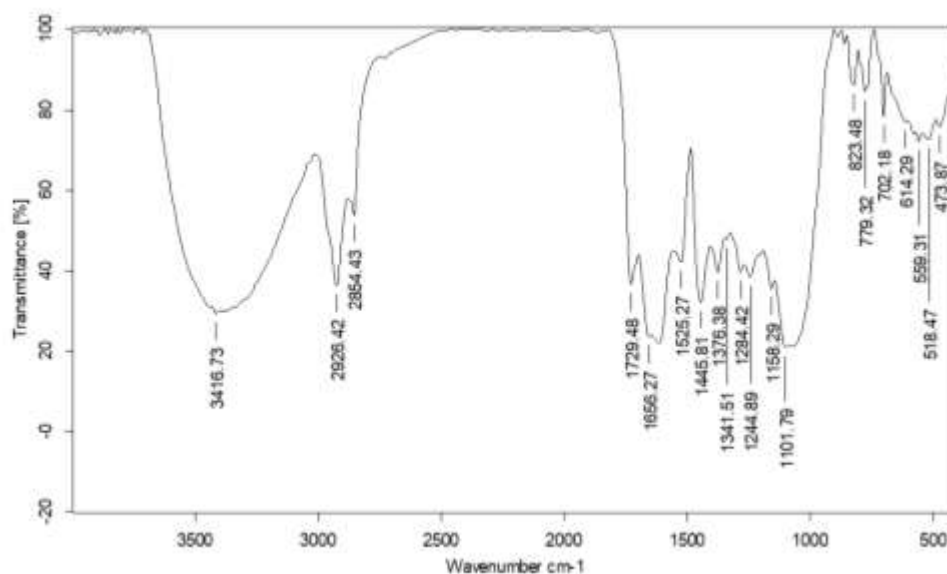


Figure 1: FTIR Spectrum analysis of stamens of *Mesua ferrea* L.

Table 2: Structural features of the *C. tamala* T. Nees & Eberm. unripe fruits by FTIR spectrum

Wave numbers (cm ⁻¹)	Functional Group	Functional Group Name	Vibrations
3433.07	O-H	Hydroxy	Stretch
2922.86	C-H	Lipids	Asymmetric stretch
2852.33	C-H	Fatty acids, Lipids, Proteins	Symmetric stretch
1639.64	C=O	Ketone	Stretch
1459.37	C=C	Aromatic ring	Stretch
1381.85	O-H	Alcohol	Bend
1048.78	PO ₃	Phosphate ion	Stretch

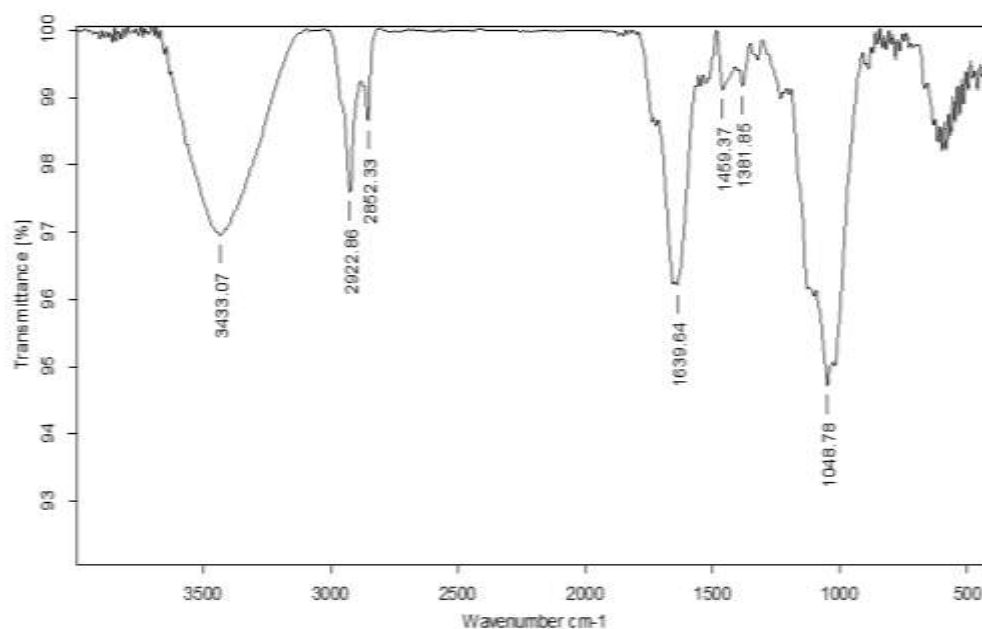
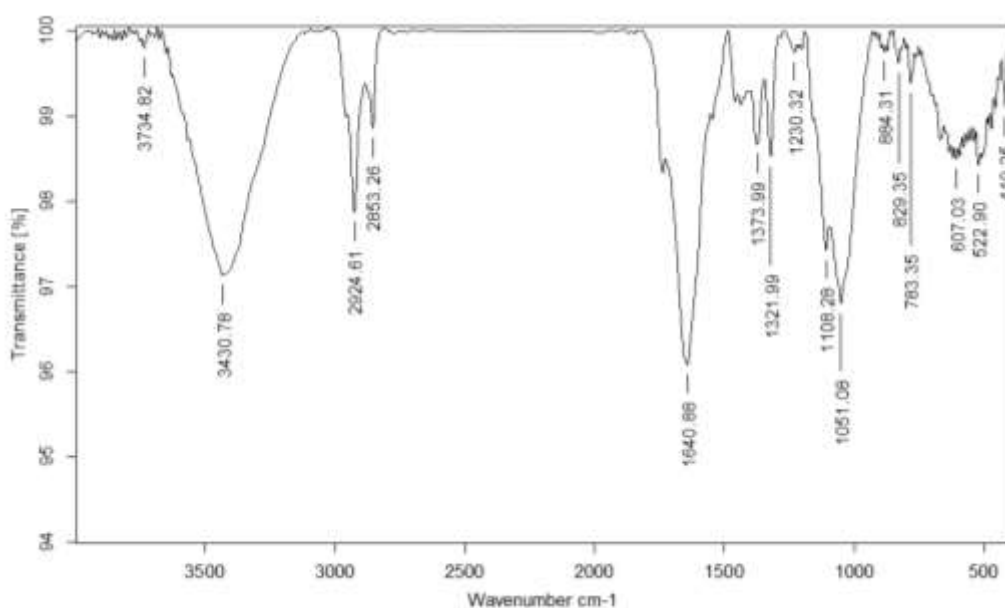


Figure 1: FTIR Spectrum analysis of unripe fruits of *Cinnamomum tamala* T. Nees & Eberm.

Table 3: Structural features of the *D. pentagyna* Roxb. fruits by FTIR spectrum

Wave numbers (cm ⁻¹)	Functional Group	Functional Group Name	Vibrations
3734.82	O-H	Hydroxy	Stretch, Non-bonded
3430.78	O-H	Hydroxy	Stretch, Bonded
2924.61	C-H	Lipids	Asymmetric stretch
2853.26	C-H	Fatty acids, Lipids, Proteins	Symmetric stretch
1640.88	C=O	Ketone	Stretch
1373.99	O-H	Alcohol	Bend
1321.99	C-O	Acid	Stretch
1230.32	C-O	Acid	Stretch
1108.28	C-N, C-H	Amine, Alkyl halide	Stretch
1051.08	PO ₃	Phosphate ion	Stretch
884.31	=C-H	Alkene	Bend
829.35	=C-H	Alkene	Bend
783.35	=C-H	Alkene	Bend
607.03	C-I, C-Cl	Alkyl halide	-
522.90	C-I, C-Cl	Alkyl halide	-

**Figure 2: FTIR Spectrum analysis of fruits of *Dillenia pentagyna* Roxb.**

GC-MS analysis

The bioactive constituents found in the soxhleted methanolic extract of *Mesua ferrea* L. stamens, unripe fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb. are detailed in Tables 5-7, listing their names, molecular formulas, retention times, and quantities. *Mesua ferrea* L. stamens, upon GC-MS

analysis, revealed the presence of 12 compounds (table 4 and figure 4), with 3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo (28.192%), Spathulenol (18.958%), and 2-[4-methyl-6-[2,6,6-trimethylcyclohex-1-enyl]hexa-1,3,5-trienylcyclohex-1-en-1-carboxaldehyde (12.670%) identified as the top three major compounds. The GC-MS chromatogram of *C. tamala* T. Nees & Eberm. unripe fruits (figure 5)

exhibited 8 peaks, representing 8 compounds (table 5), with Palmitin,1-mono (57.468%), Stearin,2-mono (34.529%), and Anodendroside E 2 (4.618%) as the top three major compounds. Methanolic extract of *D. pentagyna* Roxb. fruits revealed 4 compounds (table 6 and figure 6), highlighting (E)-13-Docosenoic acid (86.530%) followed by 15-Tetracosenoic acid, methyl ester, (Z)- (12.614%) as major components. Five specific biologically active compounds, namely 2-Propanone, 1-hydroxy ($C_3H_6O_2$), Hexanoic acid, 2-methyl ($C_7H_{14}O_2$), 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2b]oxiren5(1aH0one, 2,7,9,19tetrakis

s(acetyloxy)decahydro-3,6,8,8,10a pentamethyl ($C_{28}H_{38}O_{11}$), Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo ($C_{12}H_{16}O_5$), Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester ($C_{16}H_{26}O_2$), are identified as potential biomarkers. These compounds are present in the authentic drug (*Mesua ferrea* L. stamens) but absent in the adulterants (*Cinnamomum tamala* T. Nees & Eberm. unripe fruits and *Dillenia pentagyna* Roxb. fruits), suggesting their utility in detecting adulteration. Notably, the adulterants contain numerous other biologically active compounds like flavonoids and phenolic acids, indicating their potential use as distinct drugs.

Table 4: Biologically active chemical compounds of methanol extract from *M. ferrea* L. stamens.

Peak	Name	Molecular formula	R. Time	Peak area %
1	2-Propanone, 1- hydroxy	$C_3H_6O_2$	4.84	2.186
2	Hexanoic acid, 2- methyl	$C_7H_{14}O_2$	6.31	5.293
3	1b, 4a- Epoxy-2H-cyclopenta [3,4] cyclopropa [8,9] cycloundec[1,2-b] oxiren-5 (1aH0-one, 2,7,9,19- tetrakis (acetyloxy) decahydro-3,6,8,8,10a- pentamethyl	$C_{28}H_{38}O_{11}$	17.15	4.168
4	3-Furanacetic acid, 4- hexyl-2,5-dihydro-2,5-dioxo	$C_{12}H_{16}O_5$	17.29	28.192
5	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienylcyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	17.64	1.947
6	Formic acid,3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	$C_{16}H_{26}O_2$	17.78	8.589
7	Spathulenol	$C_{15}H_{24}O$	18.05	18.958
8	1-Heptatriacotanol	$C_{37}H_{76}O$	18.46	3.178
9	1-Heptatriacotanol	$C_{37}H_{76}O$	18.58	7.073
10	2-[4-methyl-6-[2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienylcyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	18.71	12.670
11	1-Heptatriacotanol	$C_{37}H_{76}O$	19.03	5.661
12	1-Heptatriacotanol	$C_{37}H_{76}O$	19.16	2.080

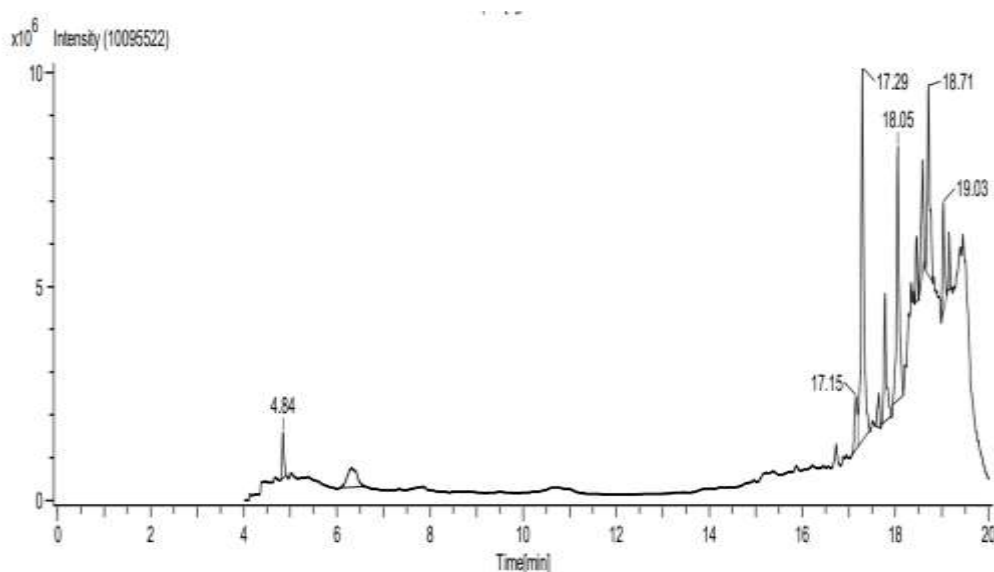


Figure 3: GC-MS chromatogram of stamens of *Mesua ferrea* Linn.

Table 5: Biologically active chemical compounds of methanol extract from *C. tamala* T. Nees & Eberm. unripe fruits

Peak	Name	Molecular formula	R. Time	Peak area %
1	Lycophyll	$C_{40}H_{56}O_2$	20.54	1.973
2	Anodendroside E 2	$C_{30}H_{38}O_{11}$	21.77	4.618
3	3'H-Cycloprop (1,2)-5-cholest-1-en-3-one,1'carboethoxy-1'-cyano-1,2-dihydro	$C_{32}H_{49}NO_3$	22.03	0.657
4	1-Palmito-2,3-distearin	$C_{55}H_{106}O_6$	30.3	0.258
5	Dimethyl triacontanedioate	$C_{32}H_{62}O_4$	30.49	0.207
6	Olein, 3-palmito-2-steoro-1	$C_{55}H_{104}O_6$	32.25	0.285
7	Palmitin, 1-mono	$C_{19}H_{38}O_4$	36.02	57.468
8	Stearin, 2-mono	$C_{21}H_{42}O_4$	38.5	34.529

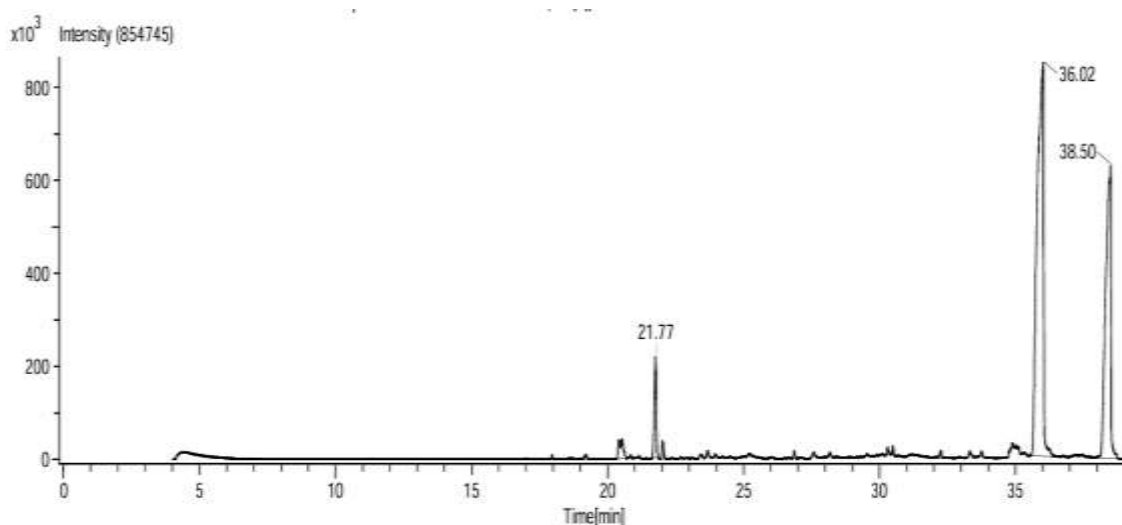
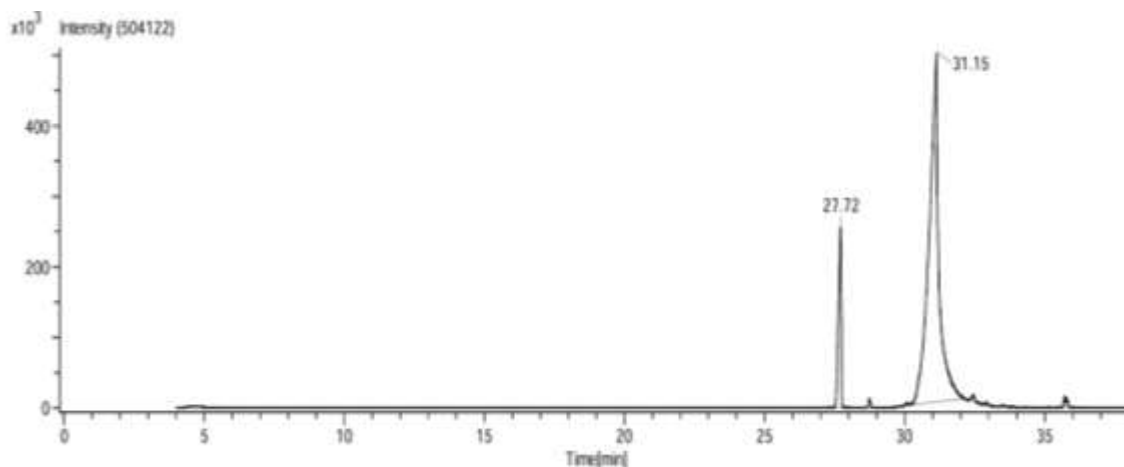


Figure 4: GC-MS chromatogram of unripe fruits of *Cinnamomum tamala* T. Nees & Eberm.

Table 6: Biologically active chemical compounds of methanol extract from *D. pentagyna* Roxb. fruits

Peak	Name	Molecular formula	R. Time	Peak area %
1	15-Tetracosenoic acid, methyl ester, (Z)-	C ₂₅ H ₄₈ O ₂	27.72	12.614
2	(E)-13-Docosenoic acid	C ₂₂ H ₄₂ O ₂	31.15	86.530
3	Cholest-3-eno [3,4-b-naphthalene, 1'-nitro]	C ₃₅ H ₄₉ NO ₂	35.72	0.475
4	9-Octadecenoic acid (Z)-, 2-butoxyethyl ester	C ₂₄ H ₄₆ O ₃	35.80	0.379

**Figure 5: GC-MS chromatogram of fruits of *Dillenia pentagyna* Roxb.**

DISCUSSION

FTIR and GC-MS are analytical techniques which can be used to detect adulteration in medicinal plants [6], [7]. FTIR identifies functional groups and molecular bonds in plant extracts and compares the spectra of authentic and adulterated samples whereas GC-MS separates and identifies volatiles and semi volatile compounds in plant extracts and simultaneously compares chromatograms and mass spectra of authentic and adulterated samples. The FTIR analysis of the stamens of *Mesua ferrea* L., unripe fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb. revealed the presence of carbonyls, amines, acids, lipids, alkenes, hydroxyls, and halogens. This indicates that all three plant samples possess significant therapeutic potential. Within the fingerprint zone (1200 to 700 cm⁻¹), FTIR spectroscopy identified unique

spectral patterns that facilitate the differentiation of *Mesua ferrea* L. from potential adulterants. Specifically, *C. tamala* exhibited a distinctive peak at 1048 cm⁻¹, and *D. pentagyna* showed characteristic absorptions at 884 cm⁻¹ and 1051 cm⁻¹ distinguishing them from each other and from *Mesua ferrea* L. The absence of absorbance between 2220-2260 cm⁻¹ indicates that none of the samples contain toxic cyanide compounds. The GC-MS analysis further differentiated the genuine *Mesua ferrea* L. from its adulterants, revealing phytochemicals unique to *Mesua ferrea* L. that were absent in *C. tamala* and *D. pentagyna*. The biological activities [8], [9] observed are attributable to the specific compounds identified in *Mesua ferrea* L. through GC-MS analysis, highlighting the significance of these phytochemicals in driving the plant's therapeutic effects. Combining FTIR and GC-MS provides a comprehensive approach to detect adulteration in

medicinal plants. These findings highlight the potential of FTIR and GC-MS analyses as reliable tools for identifying herbal adulterants. The spectral features act as biomarkers, providing a clear differentiation between authentic plant and adulterated samples. By using these techniques researchers and control professionals can ensure authenticity and safety. The implications of this study are significant for the field of herbal medicine, as the ability to distinguish genuine *Mesua ferrea* L. from its adulterants confirms the efficacy of herbal formulations. This research contributes to the development of quality control protocols, safeguarding consumers against sham practices in the herbal market. Future research could focus on expanding the spectral library to include a broader range of plant species and potential adulterants. Integrating FTIR and GC-MS with other analytical techniques, such as HPLC, could enhance the accuracy of phytochemical profiling. Investigating the biological activities of the identified compounds may further validate their therapeutic potential, paving the way for novel drug development based on genuine plant viz. *Mesua ferrea* L. and its related species.

CONCLUSION

This study demonstrates the efficacy of FTIR and GC-MS analyses in differentiating *Mesua ferrea* L. from its adulterants, *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. The distinct spectral patterns identified by FTIR and the unique phytochemical profiles obtained through GC-MS serve as reliable biomarkers for authenticating *M. ferrea* L. These findings underscore the importance of advanced spectrochemical techniques in ensuring the quality and safety of herbal products, providing a valuable framework for the identification and prevention of adulteration in the herbal medicine industry. Continued research in this area could significantly

enhance the development of standard protocols for herbal product authentication, ensuring consumer trust and the integrity of traditional medicine.

ABBREVIATION USED:

GC MS: Gas chromatography-mass Spectrometry;
FT-IR: Fourier transform- infrared.

CONFLICT OF INTERESTS:

The authors declared no conflict of interests.

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