International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2024; 12(2): 16-23
Received: 10-01-2024
Accepted: 10-02-2024

Liviya Gaikwad
Department of Botany, The Institute of Science, Dr. Homi Bhabha State University. 15, Madame Cama Road, Fort, Mumbai, Maharashtra, India

Aparna Saraf
Department of Botany, The Institute of Science, Dr. Homi Bhabha State University. 15, Madame Cama Road, Fort, Mumbai, Maharashtra, India

Corresponding Author:
Liviya Gaikwad
Department of Botany, The Institute of Science, Dr. Homi Bhabha State University. 15, Madame Cama Road, Fort, Mumbai, Maharashtra, India

Spectral fingerprint analysis of *Mesua ferrea* Linn. and its adulterants using FTIR and GC-MS techniques

Liviya Gaikwad and Aparna Saraf

DOI: https://doi.org/10.22271/flora.2024.v12.i2a.926

Abstract
Nagkesar, the stamens of *Mesua ferrea* Linn. is usually substituted with unripe fruits of *Cinnamomum wightii* Meisn. and buds of *Ochrocarpus longifolius* Benth and Hook f. knowingly and unknowingly in the markets of India. The present study was carried out to develop FTIR and GC-MS fingerprints to distinguish *M. ferrea* Linn. from its adulterants. Powdered plant samples were subjected to Fourier transform infrared (FTIR) spectrum and GC-MS analysis analysis. The FTIR spectrum confirmed the presence of various functional groups like alcohols, phenols, alkanes, alkenes, carbonyls, alkyl halides, and aromatic compounds in all plant samples with peaks at different absorption, respectively. The FTIR analysis reveals distinct spectrum patterns helping to distinguish the adulterants. The presence of 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, 2-b] oxiren-5 (1aH0-one, 2, 7, 9, 19-tetrakis(acetyloxy) decahydro-3, 6, 8, 8, 10a- pentamethyl ($C_{28}H_{38}O_{11}$), Furanacetic acid, 4- hexyl-2, 5-dihydro-2, 5-dioxo ($C_{12}H_{16}O_5$), Formicacid, 3, 7, 11-trimethyl-1, 6, 10-dodecatrien-3-yl ester ($C_{16}H_{26}O_2$) in the stamens of *Mesua ferrea Linn.* were determine through GC-MS analysis. These compounds can thus aid in detecting adulteration as they are absent in the adulterants. The spectral fingerprints showed that there is no relation between the genuine drug and the adulterants. The FTIR and GC-MS chromatogram exhibited enough variations for the identification of adulteration.

Keywords: Adulteration, nagkesar, *Mesua ferrea* linn., FTIR, gc-ms, biomarker, *Cinnamomum wightii* meisn., spectral fingerprints, *Ochrocarpus longifolius* benth and hook f.

Introduction
Since the Paleolithic period, man relies on nature for providing abundant bioresources and one of them is medicinal plants. From fighting diseases to achieving perfect health, Modern man is expansively using these medicinal plants. The phytoconstituents present gives them their uniqueness and properties making them a true treasure. One of the epitomes is *Mesua*, a large genus consisting of about 48 species of which *M. ferrea* L. is the most investigated one. It is an endangered species in India and a native of tropical Sri Lanka. It is also the official tree of Tripura. Commonly called Cobra's saffron (English) and Nagakeshara (Hindi) [1].
In Ayurveda, 'Nagkesar' is a key medication. *Mesua ferrea* L's stamen is the true source. The presence of steroids, terpenoids, and, volatile oil components are indicative of the therapeutic effects of *M. ferrea* Linn. stamens. *M. ferrea* Linn. stamens possess significant bactericidal and antioxidant activities [2]. However, because the suppliers are unaware of it, market samples are adulterated with unripe fruits of *Cinnamomum wightii* Meisn. and buds of *Ochrocarpus longifolius* Benth and Hook f. These are sold as 'Kala nagkesar' and 'Ratan nagkesar' in the market, respectively. Substituted product has no relation with the genuine drug, and may or may not have any phytoconstituents [3, 4]. Therefore, developing biomarkers would help in differentiating between genuine and adulterated plants.
The objective of this present study was to develop FTIR and GC-MS fingerprints of *M. ferrea* L., *O. longifolius* Benth and Hook f., and *C. wightii* Meisn. to distinguish *M. ferrea* Linn. from its adulterants.

Materials and Methods
Collection and authentication of plant materials
The flowers of *Mesua ferrea* L. were collected in the month of February from Veermata Jijabai Bhosale Udyan, Mumbai. The unripe fruits of *Cinnamomum wightii* Meisn. and flower buds of *Ochrocarpus longifolius* Benth and Hook f. were procured in the month of May and June from a local botanical garden in Mira Road, Mumbai. The procured plant sample *Mesua ferrea* Linn was identified and authenticated at Blatter Herbarium, St. Xavier's College, Fort, Mumbai.

The voucher specimen number given was NDG-2259.

Preparation of powder

Collected flowers of *Mesua ferrea* L. unripe fruits of *Cinnamomum wightii* Meisn., and flower buds of *Ochrocarpus longifolius* Benth and Hook f. were shade dried for one week. Stamens were then separated from the flowers of *Mesua ferrea* Linn. and powdered in a mixer blender. Shade-dried unripe fruits of *Cinnamomum wightii* Meisn. and flower buds of *Ochrocarpus longifolius* Benth and Hook f. were also ground mechanically.

FTIR spectrum analysis

Powdered plant samples were subjected to Fourier transform infrared (FTIR) spectrum analysis to identify the characteristic functional groups present. The FTIR analysis of samples was carried out in Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Mumbai. The IR spectrums were obtained using Bruker, Germany Vertex 80 FTIR System with 3000 Hyperion Microscope. The samples were scanned from 4000 to 440 cm^{-1}. The peak values of the plant materials were recorded.

GC-MS analysis

The GC-MS analysis of soxhleted methanolic extract of stamens of *Mesua ferrea* L., unripe fruits of *Cinnamomum wightii* Meisn., and flower buds of *Ochrocarpus longifolius* Benth and Hook f. was performed in Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. GC-MS analysis investigates the presence of active constituents and chemical composition present. Agilent 7890 instrument for GC and Joel Accu TOF GCV instrument for MS were used. The mixture of solvents involved toluene, chloroform, ethanol, and ethyl acetate. The inert gas helium (99.999%) was used as carrier gas with a flow rate of 1 ml/min. HP5 column with a specification length of 30 mm, internal diameter of 0.32 mm, film of 0.25 mm, and temperature limit of -60 °C to 325 °C (350 °C) was used. The run time of GC was 35 minutes. The oven temperature was raised from 70 °C up to 280 °C with the rate of 8 °C per min rise in temperature. The sample size of 4µl was injected through the injector. The MS was taken at 70eV. The identification of compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds in their library and the name, molecular weight, and structure were probably determined.

Results

FTIR analysis

The FTIR spectrum revealed the presence of 11 functional groups from the powdered sample of stamens of *Mesua ferrea* L.. Table 1 and Figure 1 demonstrate the FTIR spectrum of the stamens of *M. ferrea* L.. The absorption band at 3416.73 cm^{-1} is assigned to the H- bonded and O-H stretching vibration. The peaks at 2926.42 and 2854.43 cm^{-1} are due to asymmetric and symmetric stretching of saturated (sp^3) carbon respectively. The band at 1729.48 cm^{-1} attributes to C=O stretching associated with the carbonyl skeletal mode of the plant sample. Some other groups such as aromatic compounds, phenols or tertiary alcohols, acids, alkenes, alkyl halide phosphate ions, and halogen compounds are absorbed at 15252.27, 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, 518.47 cm^{-1} respectively.

Table 1: Structural features of the *Mesua ferrea* Linn. *stamens* by FTIR spectrum

Wave numbers (cm^{-1})	Functional Group	Functional Group Name	Vibrations
3416.73	O-H	Hydroxyl	Stretch
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	Amine	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	-

The FTIR spectrum was used to identify the functional group present in unripe fruits of *Cinnamomum wightii* Meisn. based on the peak value in the region of infrared radiation. The FTIR spectrum of *Cinnamomum wightii* Meisn.unripe fruits is depicted in Figure 2 and Table 2. A strong absorption band was observed at 3420.25 cm^{-1} due to the presence of bonded H/ O-H stretch. The C-H asymmetrical stretching methylene group was observed at 2920.23 cm^{-1}. The band observed at 2851.27 cm^{-1} represents the C-H symmetric stretching of methylene groups in aliphatic compounds. Absorbance at 1735.10 cm^{-1} shows the presence of (C=O stretch) carboxyl compounds. Bands at 1519.26, 1446.62 cm^{-1} (C=C stretch), 1320.93, 1380.44 cm^{-1} (O-H bend), 1616.57 cm^{-1} (C=O stretch), 1320.93, 1248.24 cm^{-1} (C-O stretch), 1158.67, 1105.82 cm^{-1} (C-N stretch), 893.01, 829.50, 783.13 cm^{-1} (=C-H bend), 667.12, 609.96, 559.92, 518.07 cm^{-1} (C-I/C-Cl) was detected.

Table 2: Structural features of *C. wightii* Meisn. *Unripe fruits* by FTIR spectrum

Wave numbers (cm^{-1})	Functional Group	Functional Group Name	Vibrations
3420.25	O-H	Hydroxyl	Stretch
2920.23	C-H	Lipids	Asymmetric stretch
2851.27	C-H	Fatty acids, Lipids, Proteins	Symmetric stretch
1735.10	C=O	Carbonyl	Stretch
1519.26	C=C	Aromatic ring	Stretch
1446.62	C=C	Aromatic ring	Stretch
1320.93	O-H	Phenol or tertiary alcohol	Bend
1380.44	O-H	Phenol or tertiary alcohol	Bend
1616.57	C=O	Ketone	Stretch
1320.93	C-O	Acid	Stretch
1248.24	C-O	Acid	Stretch
1158.67	C-N	Amine	Stretch
1105.82	C-N	Amine	Stretch
893.01	=C-H	Alkene	Bend
829.50	=C-H	Alkene	Bend
783.13	=C-H	Alkene	Bend
609.96	C-I, C-Cl	Alkyl halide	-
559.92	C-I, C-Cl	Alkyl halide	-
518.07	C-I, C-Cl	Alkyl halide	-

The powdered buds of *Ochrocarpus longifolius* Benth and Hook f. are passed into the FTIR spectroscopy (figure 3) and the functional groups (table 3) of the plant material are separated based on the peak values. A broad absorption band at 3410.29 indicates a hydrogen bond. This band confirms the existence of hydrate (H_2O), hydroxyl (-OH), ammonium, or amino. A narrow band at 2927.21 exhibits saturated aliphatic compounds. Absorbance at 1735.90 describes simple carbonyl compounds such as ketones, aldehydes, esters, or carbonyls. A C=O stretch indicating the presence of a ketone compound is observed due to a strong intensity band at 1615.75. A set of absorption bands at 1524.71 and 1441.45 attributed to aromatic rings. Bands at 1379.41, 1317.73, and 1282.57 are assigned to the O-H bend and C-O stretch, respectively. The presence of tertiary amine was distinguished due to the absorption bands at 1203.66 and 1160.45. An intense band occurs at 1058.48 corresponding to PO_3 stretch vibration indicating the presence of phosphate ion. Multiple bands at 857.83, 828.00, 768.66, 700.20, and 664.a53 confirm the presence of alkenes. The peaks at 664.53, 560.25, and 514.74 are assigned to halogen compounds.

Table 3: Structural features of *O. longifolius* Benth and Hook f. buds by FTIR spectrum

Wave numbers (cm^{-1})	Functional Group	Functional Group Name	Vibrations
3410.29	O-H	Hydroxyl	Stretch
2927.21	C-H	Lipids	Asymmetric stretch
1735.90	C=O	Carbonyl	Stretch
1615.75	C=O	Carbonyl	Stretch
1524.71	C=C	Aromatic ring	Stretch
1441.45	C=C	Aromatic ring	Stretch
1379.41	O-H	Phenol or tertiary alcohol	Bend
1317.73	O-H	Phenol or tertiary alcohol	Bend
1282.57	C-O	Acid	Stretch
1203.66	C-N	Amine	Stretch
1160.45	C-N	Amine	Stretch
1058.48	PO_3	Phosphate ion	Stretch
857.83	=C-H	Alkene	Bend
828.00	=C-H	Alkene	Bend
768.66	=C-H	Alkene	Bend
700.20	=C-H	Alkene	Bend
664.53	=C-H	Alkene	Bend
664.53	C-I, C-Cl	Alkyl halide	-
560.25	C-I, C-Cl	Alkyl halide	-
514.74	C-I, C-Cl	Alkyl halide	-

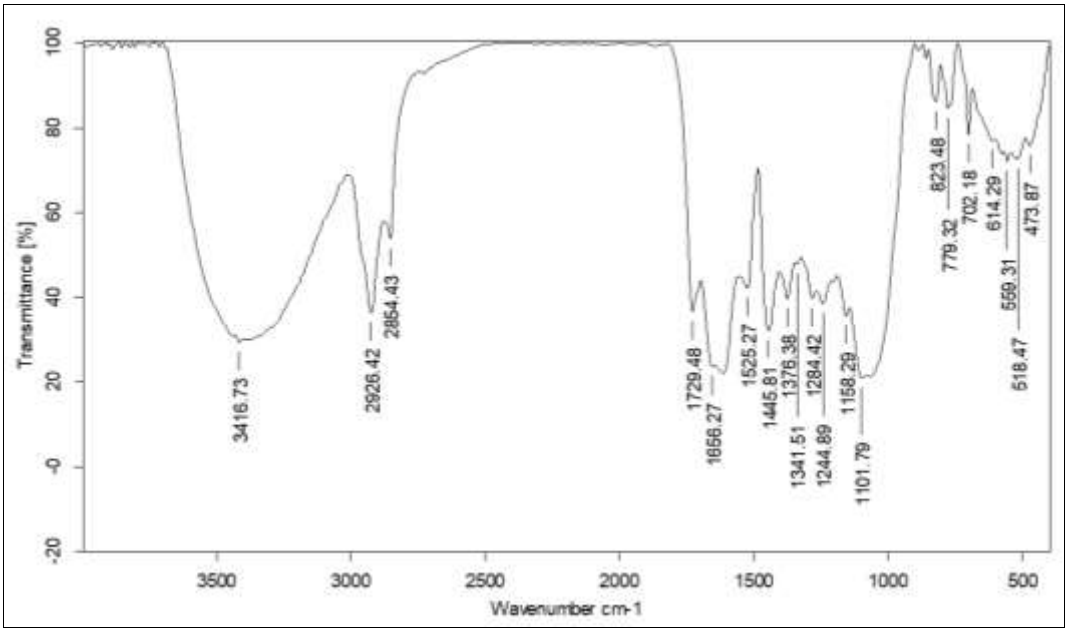

Fig 1: FTIR Spectrum analysis of *Mesua ferrea* Linn. Stamens

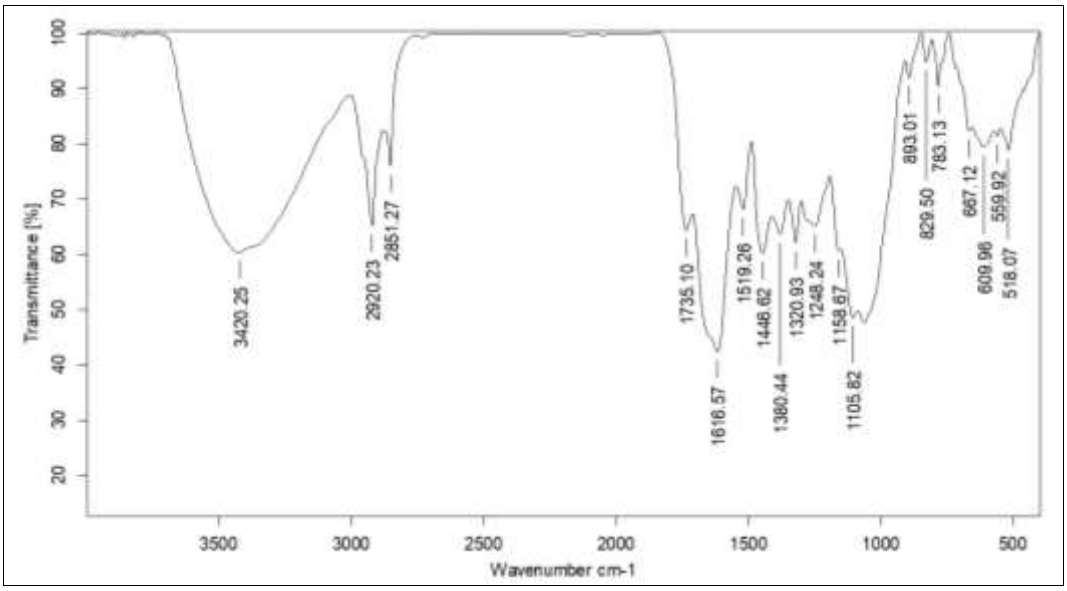

Fig 2: FTIR Spectrum analysis of *Cinnamomum wightii* Meisn.unripe fruits

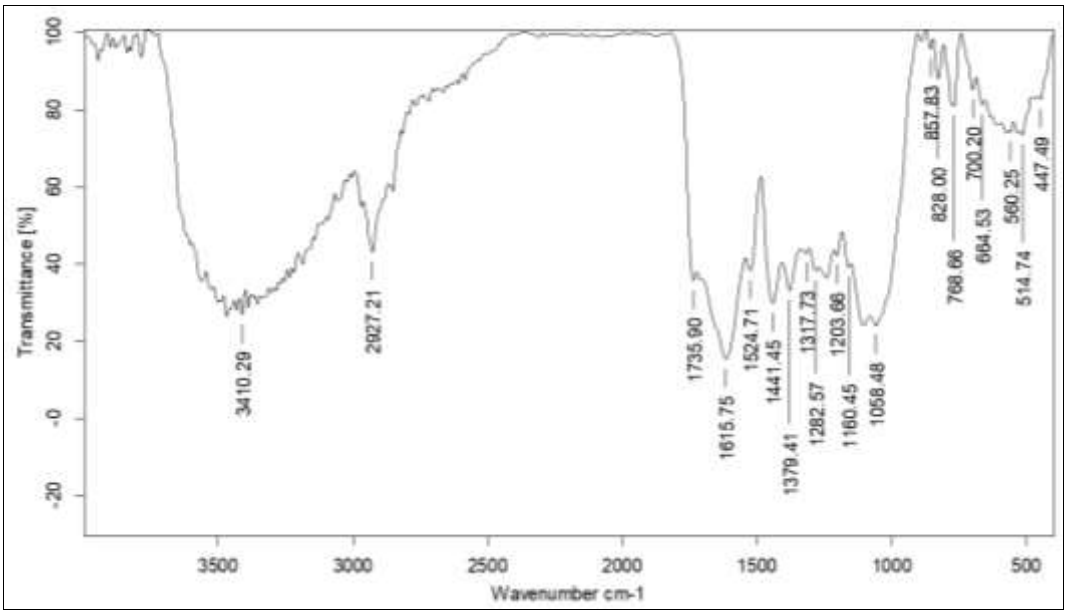

Fig 3: FTIR Spectrum analysis of *Ochrocarpus longifolius* Benth and Hook f. buds

GC-MS analysis

The bioactive compounds present in soxhleted methanolic extract of stamens of *Mesua ferrea* Linn., unripe fruits of *Cinnamomum wightii* Meisn., and buds of *Ochrocarpus longifolius* Benth and Hook f. are shown in Tables 4-6 respectively. Figure 4, figure 5 and figure 6 represents GC-MS chromatograms of *Mesua ferrea* Linn., unripe fruits of *Cinnamomum wightii* Meisn., and buds of *Ochrocarpus longifolius* Benth and Hook f. respectively. The name, molecular formula, retention time and the amount of these bioactive compounds were ascertained. GC-MS analysis of *Mesua ferrea* Linn. revealed the presence of 12 compounds (table 4). Based on abundance, 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-enylhexa-1, 3, 5-trienylcyclohex-1-en-1-carboxaldehyde (12.670%) were the three top major compounds present in the methanolic extract of *Mesua ferrea* Linn. stamens. GC- MS chromatogram of methanolic extract of *C. wightii* Meisn. unripe fruits showed 28 peaks (table 5) indicating the presence of 28 compounds with 1, 6-Octadien-3-ol, 3, 7-dimethyl- (18.813%), γ-Cadinene (12.575%) and tau-Cadinol (8.472%) as the top three major compounds. Twenty- nine compounds were identified in the methanolic extract of *O. longifolius* Benth and Hook f. buds (table 6) and exhibited the presence of Eremophilene (13.651%), 1-Heptatriacotanol (10.212%), and 8, 11-Octadecadienoic acid, methyl ester (9.304%) as major components.

Table 4: Biologically active chemical compounds of methanol extract from *M. ferrea* Linn. 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-enylhexa-1, 3, 5-trienylcyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	17.64	1.947
6	Formicacid, 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-enylhexa-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

Table 5: Biologically active chemical compounds of methanol extract from *C. wightii* Meisn. unripe fruits.

Peak	Name	Molecular formula	R. Time	Peak area %
1	trans-3-Caren-2-ol	$C_{10}H_{16}O$	8.62	0.757
2	Docosahexaenoic acid, 1, 2, 3-propanetriyl ester	$C_{69}H_{98}O_6$	8.87	0.369
3	1, 6-Octadien-3-ol, 3, 7-dimethyl-	$C_{10}H_{18}O$	11.27	18.813
4	Copaene	$C_{15}H_{24}$	16.23	5.224
5	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	16.50	0.520
6	Caryophyllene	$C_{15}H_{24}$	16.70	7.718
7	1H-Cycloprop[e]azulene, 1a, 2, 3, 5, 6, 7, 7a, 7b-octahydro-1, 1, 4, 7-tetramethyl-, [1aR-(1aα, 7α, 7aβ, 7bα)]-	$C_{15}H_{24}$	16.87	0.824
8	α-Caryophyllene	$C_{15}H_{24}$	17.01	1.641
9	1H-Cycloprop[e]azulene, decahydro-1, 1, 7-trimethyl-4-methylene-, [1aR-(1aα, 4aβ, 7α, 7aβ, 7bα)]-	$C_{15}H_{24}$	17.07	1.387
10	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α, 4aα, 8aα)-	$C_{15}H_{24}$	17.17	1.663
11	α-Muurolene	$C_{15}H_{24}$	17.36	5.687
12	γ-Cadinene	$C_{15}H_{24}$	17.52	12.575
13	Naphthalene, 1, 2, 4a, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl)-, [1R-(1α, 4aα, 8aα)]-	$C_{15}H_{24}$	17.68	3.678
14	Caryophyllene oxide	$C_{15}H_{24}O$	18.07	6.400
15	Bicyclo [4.4.0] dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	$C_{15}H_{24}O_2$	18.27	1.925
16	β-Guaiene	$C_{15}H_{24}$	18.36	2.789
17	tau-Cadinol	$C_{15}H_{26}O$	18.50	8.472
18	α-Cadinol	$C_{15}H_{26}O$	18.59	5.858
19	Sericealactone, deoxy-	$C_{16}H_{20}O_4$	18.70	0.700
20	1-Heptatriacotanol	$C_{37}H_{76}O$	18.84	0.774
21	1-Heptatriacotanol	$C_{37}H_{76}O$	19.07	3.177
22	1-Heptatriacotanol	$C_{37}H_{76}O$	19.23	1.482
23	2, 2-Dimethyl-6-methylene-1-[3, 5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	$C_{14}H_{24}O_4$	19.32	1.525
24	2-[4-methyl-6-(2, 6, 6-trimethylcyclohex-1-enyl)hexa-1, 3, 5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	19.59	1.664
25	1-Heptatriacotanol	$C_{37}H_{76}O$	19.66	1.773
26	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl) cyclopropyl] methyl] cyclopropyl] methyl]-, methyl ester	$C_{25}H_{42}O_2$	19.86	0.741
27	Estra-1, 3, 5(10)-trien-17β-ol	$C_{18}H_{24}O$	20.20	1.080
28	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	20.86	0.773

Table 6: Biologically active chemical compounds of methanol extract from *O. longifolius* Benth and Hook f. buds

Peak	Name	Molecular formula	R. Time	Peak area %
1	Cobalt, nonacarbonyl [μ3-(oxophenylethylidyne)] tri-, triangulo	$C_{17}H_5Co_3O_{10}$	10.3	2.122
2	2, 4, 6, 8, 10-Tetradecapentaenoic acid, 9a-(acetyloxy)-1a, 1b, 4, 4a, 5, 7a, 7b, 8, 9, 9a-decahydro-4a, 7b-dihydroxy-3-(hydroxymethyl)-1, 1, 6, 8-tetramethyl-5-oxo-1H-cyclopropa [3, 4]	$C_{36}H_{46}O_8$	11.10	0.385
3	Copaene	$C_{15}H_{24}$	16.21	2.690
4	9, 12, 15-Octadecatrienoic acid, 2-phenyl-1, 3-dioxan-5-yl ester	$C_{28}H_{40}O_4$	16.56	0.562
5	Caryophyllene	$C_{15}H_{24}$	16.68	1.953
6	Cholesta-8, 24-dien-3-ol, 4-methyl-, (3β, 4α)-	$C_{28}H_{46}O$	16.75	1.309
7	1H-Cycloprop[e]azulene, 1a, 2, 3, 5, 6, 7, 7a, 7b-octahydro-1, 1, 4, 7-tetramethyl-, [1aR-(1aα, 7α, 7aβ, 7bα)]-	$C_{15}H_{24}$	17.14	5.797
8	1H-Cycloprop[e]azulene, 1a, 2, 3, 5, 6, 7, 7a, 7b-octahydro-1, 1, 4, 7-tetramethyl-, [1aR-(1aα, 7α, 7aβ, 7bα)]-	$C_{15}H_{24}$	17.27	3.842
9	Eremophilene	$C_{15}H_{24}$	17.34	13.651
10	δ-Cadinene	$C_{15}H_{24}$	17.51	6.222
11	2-[4-methyl-6-(2, 6, 6-trimethylcyclohex-1-enyl) hexa-1, 3, 5-trienyl)cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	17.64	2.315
12	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	17.72	0.521
13	1-Heptatriacotanol	$C_{37}H_{76}O$	18.06	2.912
14	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	18.26	0.824
15	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	18.34	0.689
16	1-Heptatriacotanol	$C_{37}H_{76}O$	18.45	3.509
17	1-Heptatriacotanol	$C_{37}H_{76}O$	18.56	3.311
18	1-Heptatriacotanol	$C_{37}H_{76}O$	18.84	2.242
19	1-Heptatriacotanol	$C_{37}H_{76}O$	18.92	0.956
20	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	18.97	4.333
21	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	19.38	0.873
22	1-Heptatriacotanol	$C_{37}H_{76}O$	19.45	1.304
23	Sericealactone, deoxy-	$C_{16}H_{20}O_4$	19.51	7.263
24	Methyl 14-methylhexadecanoate	$C_{18}H_{36}O_2$	19.86	4.280
25	1-Heptatriacotanol	$C_{37}H_{76}O$	20.02	2.297
26	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	20.25	2.696
27	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	20.53	1.612
28	8, 11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	20.75	9.304
29	1-Heptatriacotanol	$C_{37}H_{76}O$	20.85	10.212

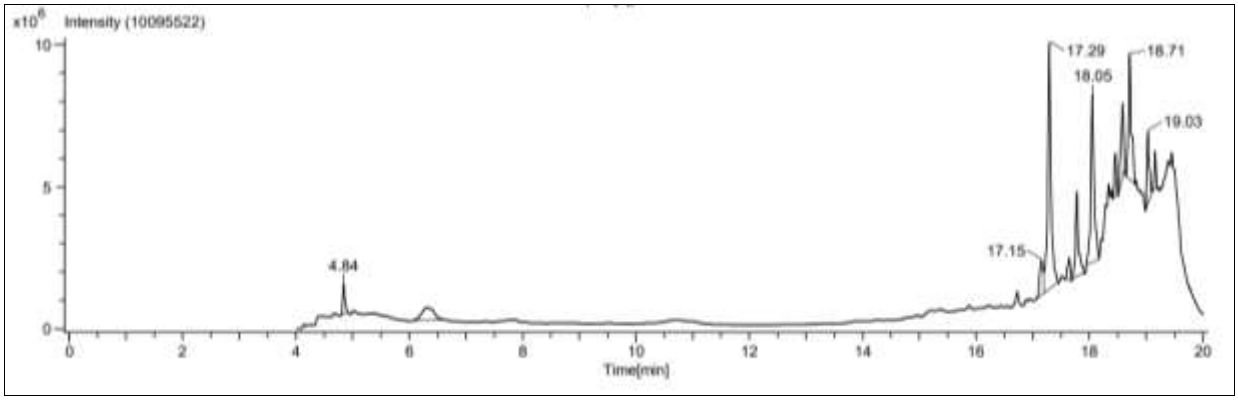

Fig 4: GC-MS chromatogram of Mesua ferrea's L. stamens

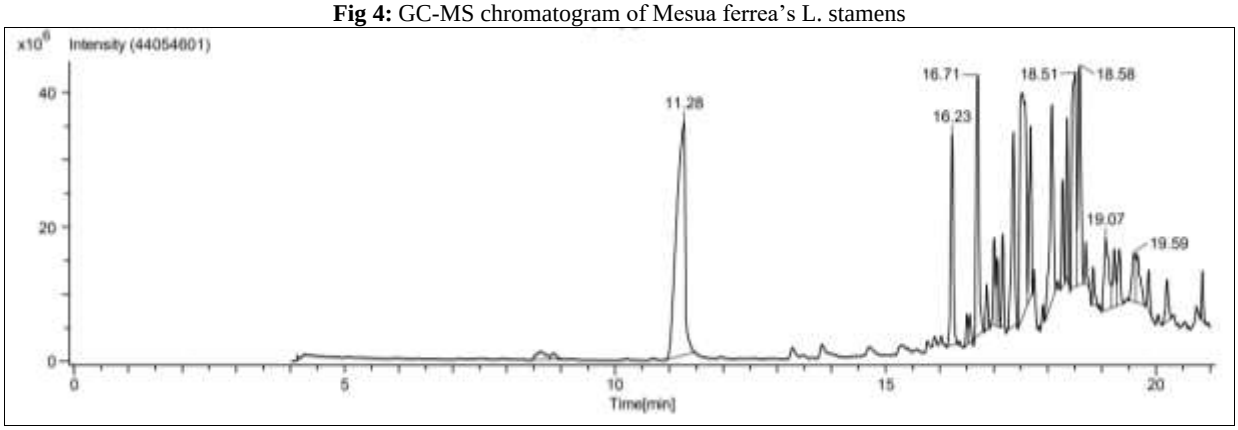

Fig 5: GC-MS chromatogram of unripe fruits of *Cinnamomum wightii* Meisn.

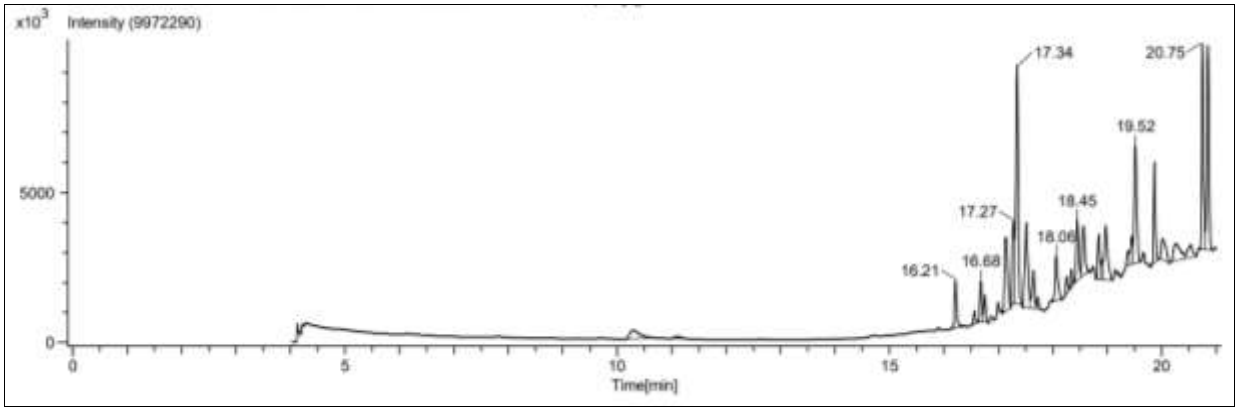

Fig 6: GC-MS chromatogram of buds of *Ochrocarpus longifolius* Benth and Hook f.

Discussions

FTIR analysis in the fingerprint zone (1200 to 700 cm^{-1}) reveals a distinct spectrum pattern helping to distinguish *M. ferrea* Linn. from the adulterants. *Cinnamomum wightii* Meisn. shows characteristic absorption at 893 cm^{-1} which is not found in *M. ferrea* Linn. and *O. longifolius* Benth and Hook f.. *Ochrocarpus longifolius* Benth and Hook f. shows characteristic absorption at 857 cm-1 and 1058 cm-1 which is not found in *M. ferrea* Linn. as well as *C. wightii* Meisn.. Therefore, these specific values of absorption peaks can be crucial when utilizing powdered samples of *M. ferrea* L., *Cinnamomum wightii* Meisn. and *Ochrocarpus longifolius* Benth and Hook f. in Ayurvedic preparations. The lack of absorbance between 2220 and 2260 cm^{-1} in the three powdered samples suggests the absence of cyanide, indicating the absence of any toxic substances [5, 6].

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, 2-b] oxiren-5 (1aH0-one, 2, 7, 9, 19-tetrakis (acetyloxy) decahydro-3, 6, 8, 8, 10a- pentamethyl ($C_{28}H_{38}O_{11}$), Furanacetic acid, 4- hexyl-2, 5-dihydro-2, 5-dioxo ($C_{12}H_{16}O_5$), Formicacid, 3, 7, 11-trimethyl-1, 6, 10-dodecatrien-3-yl ester ($C_{16}H_{26}O_2$) can act as biomarkers as these five biologically active compounds are present in genuine drug *viz*. stamens of *Mesua ferrea* Linn. whereas absent in the adulterants i.e., unripe fruits of *Cinnamomum wightii* Meisn., buds of *Ochrocarpus longifolius* Benth and Hook f.. Thus, these phyto constituents can help in detecting adulteration. The adulterants contained a large number of biologically active compounds such as flavonoids, and phenolic acids, therefore can be used as separate drugs.

Conclusion

FTIR analysis was carried out for *Mesua ferrea*'s stamen, unripe fruits of *Cinnamomum wightii* Meisn, and buds of *Ochrocarpus longifolius* Benth and Hook f. All three plant samples showed the presence of carbonyls, amines, acids, lipids, alkenes, hydroxyls, halogens thus posing a high-value therapeutic content. FTIR analysis in the fingerprint zone (1200 to 700 cm^{-1}) revealed a distinct spectrum pattern helping to distinguish *M. ferrea* Linn. from the adulterants. A large number of differences validate no relation between the genuine and the adulterated ones.

GC-MS analysis of genuine drug *Mesua ferrea* L. and adulterants *Cinnamomum wightii* Meisn, and *Ochrocarpus longifolius* Benth and Hook f. were investigated. The chromatograms obtained through analysis showed the presence of different phytochemicals in *M. ferrea* Linn. which were absent in the adulterants.

Exploratory and diagnostic analysis of FTIR and GC-MS spectra offers phytochemical insights and detection potential for the identification of herbal adulterants. These spectro chemical images act as biomarkers, hence detecting adulteration.

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

Acknowledgments: This work was supported by the Dr. Babasaheb Ambedkar Research and Training Institute under grant BANRF2021.

Conflict of Interests

The authors declared no conflict of interests.

References

1. Chahar MK, DS SK, Geetha L, Lokesh T, Manohara KP. *Mesua ferrea* L.: A review of the medical evidence for its phytochemistry and pharmacological actions. African Journal of Pharmacy and Pharmacology. 2013;7(6):211–219.
2. Barbade KD, Datar AG. Extraction, bioactivities, phytochemical investigation and *in-vivo* toxicity studies of *Mesua ferrea* L. Stamens. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7:93–97.
3. Dubey RB, Sonawat BS. Current scenario of adulterants and substitutes of medicinal plants: A review. Journal of Pharmaceutical Sciences and Innovation. 2015;4(5):247–250.
4. Ahmed S, Hasan MM. Crude drug adulteration: A concise review. World Journal of Pharmacy and Pharmaceutical Sciences. 2015;4(10):274–283.
5. Ragavendran P, Sophia D, Arul Raj C, Gopalakrishnan VK. Functional group analysis of various extracts of *Aerva lanata* (L.,) by FTIR spectrum. Pharmacologyonline. 2011;1:358–364.
6. Saraf A, Saraf A, Chaturvedi A. Phytochemical analysis and chemical fingerprinting of seeds of *Abrus precatorius* L. Chemical Science Transactions. 2018;7(1):63–70.