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## Research Article

# Assessing the Suitability of *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. as Substitutes for *Mesua ferrea* L. Stamens Using Multidimensional Analytical Approaches

Liviya Gaikwad\*, Aparna Saraf

Department of Botany, The Institute of Science, Dr. Homi Bhabha State University, 15, Madame Cama Road, Fort, Mumbai – 400032 India

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## ABSTRACT

The stamens of *Mesua ferrea* L. (Nagkesar) are highly valued for their medicinal properties but are often adulterated with the unripe fruits of *Cinnamomum tamala* T. Nees & Eberm., commonly sold as Kala Nagkesar, and the fruits of *Dillenia pentagyna* Roxb., marketed as Malabari Nagkesar, due to economic and availability factors. While leaves of *Cinnamomum tamala* T. Nees & Eberm. and leaves, bark and roots of *Dillenia pentagyna* Roxb. are documented for their distinct medicinal properties, this study aims to evaluate whether unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb. can serve as equivalent substitutes for the stamens of *Mesua ferrea* L. Advanced analytical techniques, including Field Emission Gun Scanning Electron Microscopy (FEGSEM), High-Performance Liquid Chromatography (HPLC), and High-Resolution Orbitrap Liquid Chromatograph Mass Spectrometer (HR-LCMS-Orbitrap), were used to analyse and compare the morphological and phytochemical profiles of the samples. FEGSEM revealed clear microstructural differences between the stamens of *Mesua ferrea* L. and the unripe fruits of *C. tamala* T. Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb. HPLC and HR-LCMS-Orbitrap identified unique phytochemical markers and significant variations in chemical composition. The findings confirm that the unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb. cannot replicate the properties of *Mesua ferrea* L. stamens. This study highlights the need for stringent quality control measures to prevent adulteration and ensure the authenticity and therapeutic efficacy of herbal formulations.

## INTRODUCTION

Medicinal plants have been integral to the Indian system of medicine, particularly Ayurveda, for

\*Corresponding Author: Liviya Gaikwad

Address: Department of Botany, The Institute of Science, Dr. Homi Bhabha State University, 15, Madame Cama Road, Fort, Mumbai – 400032 India

Email ✉: [liviyaigaikwad@yahoo.com](mailto:liviyaigaikwad@yahoo.com)

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millennia. They are revered not only for their therapeutic efficacy but also for their cultural and spiritual significance. Among these, *Mesua ferrea* L., known as Nagkesar, occupies a prominent position due to its diverse pharmacological activities [1]. The stamens of *Mesua ferrea* L. are renowned for their applications in managing bleeding disorders, inflammation, and microbial infections [2]. The bioactive compounds such as xanthenes, flavonoids, and essential oils underpin these therapeutic properties, offering immense potential for modern drug discovery [3].

However, increasing demand and challenges in ensuring a consistent supply of *Mesua ferrea* L. have led to the substitution or adulteration of its stamens with other botanicals [4]. In particular, the unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. (sold as Kala Nagkesar) and the fruits of *Dillenia pentagyna* Roxb. (referred to as Malabari Nagkesar) are commonly found as adulterants or substitutes in the market [2]. Although these substitutions may help address supply issues, their chemical equivalence and therapeutic viability remain insufficiently studied, thereby risking the quality and efficacy of traditional formulations.

Recent investigations have attempted to elucidate the phytochemical profiles and pharmacological potentials of *Mesua ferrea* L. [5], [6], [7], [8], [9]. A detailed analysis of the secondary metabolites present in *Mesua ferrea* L., highlighting significant variations based on environmental and processing factors was reported [10]. Similarly, a comparative evaluation of *Mesua ferrea* L. alongside its common substitutes, revealing notable discrepancies that question the assumption of therapeutic equivalence [11]. Although these studies contribute valuable insights, many focus on isolated parameters without integrating the broader phytochemical context or critically evaluating the methodological limitations.

The leaves of *Cinnamomum tamala* T. Nees & Eberm., known as Tejpatta or Indian bay leaf, are widely utilized for their culinary and medicinal properties. In traditional medicine, they are valued for managing digestive ailments, such as gas and bloating, as well as respiratory issues like asthma. Additionally, the leaves exhibit hypoglycaemic, antimicrobial, and antioxidant properties, making them useful for treating diabetes and promoting cardiovascular health [12], [13], [14].

On the other hand, *Dillenia pentagyna* Roxb. has a more limited but noteworthy role in traditional medicine. Its bark and fruits are employed for their antioxidant, antimicrobial, and antidiabetic activities [15], [16], [17]. The fruits, in particular, are consumed as a vegetable or decoction for managing diabetes and are noted for their use in treating coughs and blood dysentery in certain tribal practices [18], [19]. Nevertheless, critical comparative analyses of these substitutes vis-à-vis *Mesua ferrea* L. remain sparse. The available literature predominantly summarizes individual attributes of these plants without offering a systematic review that integrates and critically evaluates their phytochemical and pharmacological interrelationships.

While both *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. possess distinct phytochemical and therapeutic attributes, their efficacy as substitutes for *Mesua ferrea* L. is yet to be conclusively determined.

The uncontrolled substitution of *Mesua ferrea* L. with botanicals such as *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. raises concerns about the quality, safety, and therapeutic consistency of herbal formulations in Ayurvedic medicine. Without robust, standardized analytical methods to verify chemical and pharmacological equivalence, there is a significant risk of compromised efficacy in traditional



treatments. While extensive research has detailed the pharmacological properties of *Mesua ferrea* L. and the individual benefits of *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb., few studies have systematically compared their phytochemical profiles and therapeutic effects. The lack of comprehensive data on whether these substitutes can reliably mimic the bioactive properties of *Mesua ferrea* L. underscores a critical gap in the literature.

This study seeks to bridge the existing research gap by employing a multidimensional analytical approach to evaluate the authenticity and efficacy of these botanicals. The research involves the use of Field Emission Gun Scanning Electron Microscopy (FEGSEM) to rapidly identify and compare the morphological characteristics of *Mesua ferrea* L., *Cinnamomum tamala* T. Nees & Eberm., and *Dillenia pentagyna* Roxb. [20]. Detailed chemical profiles will be established using advanced chromatographic techniques, including High-Performance Liquid Chromatography (HPLC) and High-Resolution Liquid Chromatography-Mass Spectrometry-Orbitrap (HR-LCMS-Orbitrap) [21], which will enable the identification and comparison of key bioactive compounds across these species. Thus, the study assesses whether the chemical equivalence observed can translate into similar pharmacological properties, thereby evaluating the therapeutic viability of *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. as substitutes for genuine *Mesua ferrea* L. By establishing these correlations, the research aims to lay a scientific foundation for ensuring the integrity and efficacy of Ayurvedic formulations, ultimately safeguarding patient safety and enhancing treatment outcomes.

## MATERIALS AND METHODS

### Collection and authentication of plant materials

In February, the flowers of *Mesua ferrea* L. were collected from Veer Mata Jijabai Bhosale Udyan in Mumbai. The immature fruits of *Cinnamomum tamala* T. Nees & Eberm. were obtained in June from a botanical garden in Dahanu (Palghar, Maharashtra). The fruits of *Dillenia pentagyna* Roxb. were collected in June from the Dapoli Urban Bank Senior Science College, Jalgaon, Maharashtra, India. The specimens of *Mesua ferrea* L. were identified and authenticated at the Blatter Herbarium at St. Xavier's College in Fort, Mumbai, with voucher specimens assigned for reference purposes (NDG-2259). To ensure experimental robustness, authenticated reference samples served as controls, and all analyses were performed in triplicate to account for biological variability and to enhance reproducibility.

### Preparation of powder

Following collection, the plant materials were processed under standardized conditions. The flowers of *Mesua ferrea* L., immature fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb. were air-dried in the shade for one week. Following this, the stamens were separated from the flowers and ground into a fine powder using a mixer blender. Additionally, the shade-dried immature fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb. were mechanically ground.

### Field Emission Gun Scanning Electron Microscopy (FEGSEM) analysis

FEGSEM imaging of *Mesua ferrea* L. stamens, along with immature fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb., was conducted using a JOEL JSM-7600F microscope. The system featured a



scanning electron microscope (SEM) with a spatial resolution of 1.0 nm at 15 kV, and magnification capabilities ranging from Low (25X to 10,000X) to High (100X to 1,000,000X). The imaging process combined electron column and field emission gun techniques with sample preparation. The material was thoroughly dried in an oven at 40 °C for 10–12 hours, then mounted on stubs using double-sided tape. A very thin single layer of the powdered samples was applied before coating with a 10 nm layer of Iridium for FEGSEM analysis.

### High-Performance Liquid Chromatography (HPLC)

HPLC studies were performed at 30°C using an Agilent 1200 Eclipse XDB-C18 column with dimensions of 5 µm and 4.6 mm × 250 mm. The detection wavelength was set to 254 nm, the injection volume was 10 µL, and the flow rate was 1.0 mL/min, utilizing a mobile phase composed of methanol and water in a ratio of 80:20 (v/v) for compound separation. Before sample analysis, the HPLC system was calibrated using a standard mixture of analytes to ensure the accuracy and reproducibility of the results. The chromatographic data were captured and processed using the Agilent Chemstation 1100 software version. Chromatographic peaks were identified by comparing retention times and spectral data with authentic standards.

### High-Resolution Orbitrap Liquid Chromatograph Mass Spectrometer (HR-LCMS-Orbitrap)

The LC-MS analysis of Soxhlet-extracted methanolic plant samples was performed using a Q-Exactive Plus Orbitrap MS, coupled with Thermo Scientific's Xcalibur software for data acquisition and Compound Discoverer 3.2 SP1 for data processing to investigate active constituents and characterize the chemical composition. A

Hypersil GOLD column (150 x 2.1 mm, 1.9 microns; Thermo Scientific) was employed for separation. The mobile phase consisted 0.1% formic acid in Milli-Q water, and acetonitrile (ACN). The column temperature was set to 40°C, with a flow rate maintained at 0.300 mL/min over a total run time of 35 minutes. The gradient elution started with 5% ACN for the first 2 minutes, increased to 95% ACN between 20 and 25 minutes, and then returned to 5% ACN from 26 to 30 minutes. The sampler temperature was adjusted to 4°C with a wash speed of 10 µL/s. Mass spectrometry was conducted in positive and negative polarity mode over a 35-minute runtime, with full MS scans ranging from 105 to 1500 m/z at a resolution of 70,000 and an AGC target of 1e6. The dd-MS<sup>2</sup> scans spanned from 200 to 2000 m/z at a resolution of 17,500, with an isolation window of 1.5 m/z and a dynamic exclusion setting of 10 seconds. Calibration of the instrument was performed prior to each analytical session using established calibration standards. Data processing involved both targeted and untargeted approaches; peaks were identified through spectral matching against established libraries and verified using internal standards.

## RESULTS

### Field Emission Gun Scanning Electron Microscopy (FEGSEM) analysis

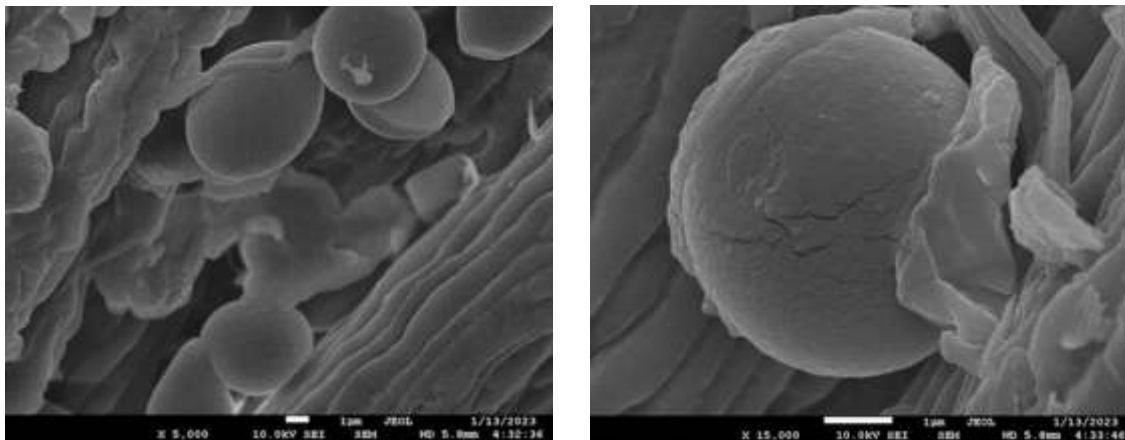
The FEGSEM analysis of *Mesua ferrea* L. stamens (figure 1) revealed smooth and globular, oval-shaped pollen grains with a fine reticulate pattern, presenting a tricolpate structure crucial for accurate species identification.

The FEGSEM analysis of the unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. (figure 2) showed a smooth, compact surface with minimal roughness. Epidermal cells were well-organized, and pollen grains were predominantly spherical to

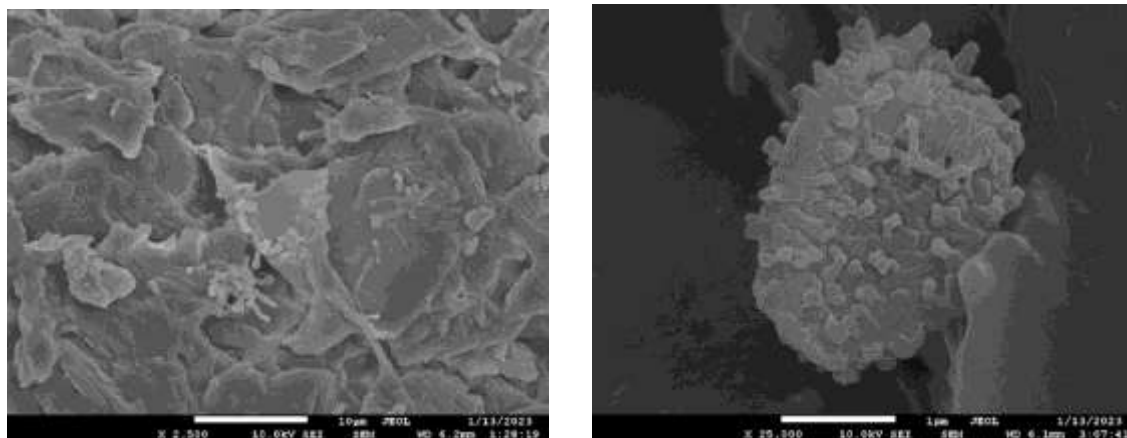


slightly ellipsoidal, displaying a reticulate exine pattern. The grains were monocolpate, with a single elongated aperture and a moderately perforated exine.

The surface of the fruits of *Dillenia pentagyna* Roxb. (figure 3) exhibited spherical to slightly ellipsoidal pollen grains with a finely reticulate surface, including small protrusions and ridges, characterized as tricolpate with three apertures.

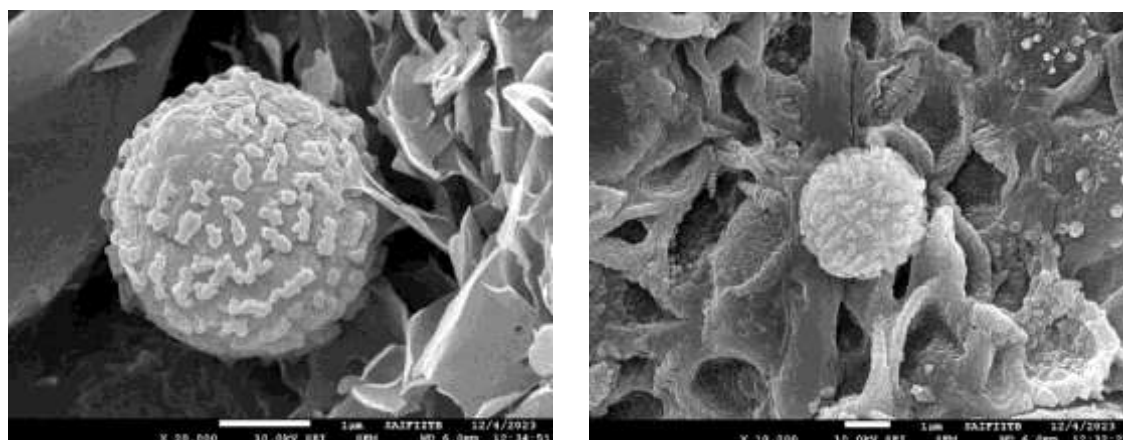


**Figure 1: Field emission scanning electron micrographs of the stamens of *Mesua ferrea* L. a- pollen grains at 5000X, b- pollen grain at 15000X**



**Figure 2: Field emission scanning electron micrographs of unripe fruits of *Cinnamomum tamala* T. Nees & Eberm.**

**c: Smooth, compact surface, with minimal roughness, d: pollen grain at 25000X**



**Figure 3: Field emission scanning electron micrographs of fruits of *Dillenia pentagyna* Roxb. e- pollen grains at 2000X, f- pollen grain at 10000X**

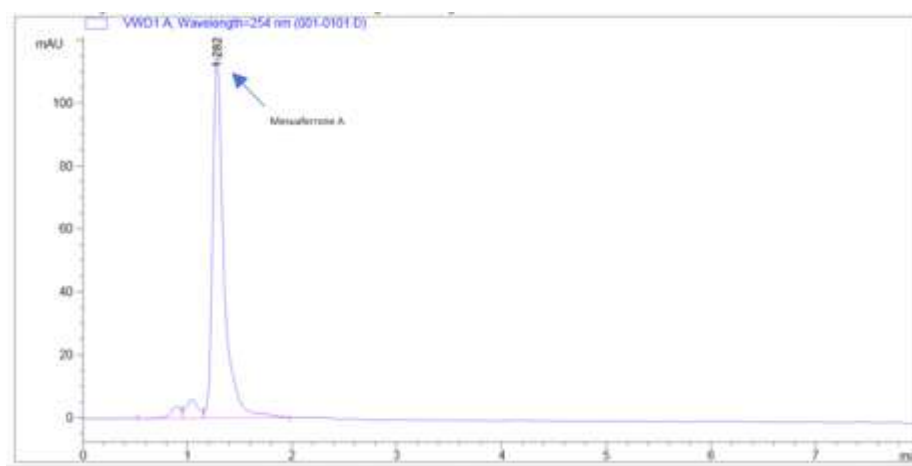
### High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) was performed on the stamens of *Mesua ferrea* L., unripe fruits of *Cinnamomum tamala* T. Nees & Eberm., and fruits of *Dillenia pentagyna* Roxb. (table 1) to evaluate their chemical constituents. 10  $\mu$ L of reference and sample solutions were injected into the liquid chromatograph, and detection was carried out at 254 nm. Mesuaferrone A, also known as Rhusflavanone [22], served as a quality control marker, presenting a distinct peak with a retention time of 1.282 minutes (figure 4). This peak was used as a benchmark for comparing the chemical profiles of genuine and adulterant plant extracts. A comparative analysis of the chemical profiles of *Mesua ferrea* L. with *C. tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. (figure 5) revealed that the second chromatographic peak for *Mesua ferrea* L. corresponded to the retention time

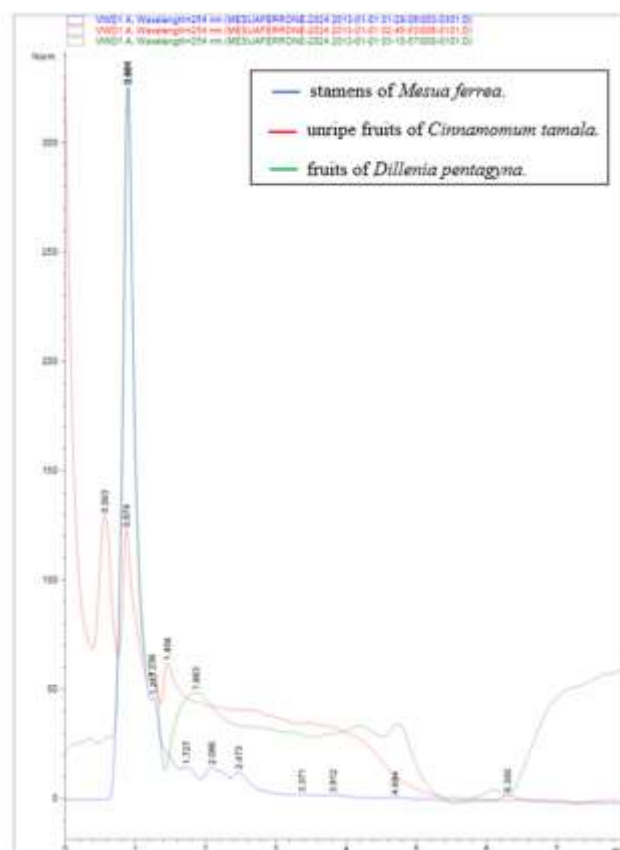
of the Mesuaferrone A reference solution. The analysis demonstrated the unique chemical compositions of the three plant species, as reflected in their characteristic retention times during HPLC analysis.

**Table 1: The retention times of stamens of *Mesua ferrea* L., unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb. by HPLC.**

| Peaks | Retention time (mins)            |                                             |                                       |
|-------|----------------------------------|---------------------------------------------|---------------------------------------|
|       | Stamens of <i>Mesua ferrea</i> . | Unripe fruits of <i>Cinnamomum tamala</i> . | Fruits of <i>Dillenia pentagyna</i> . |
| 1     | 0.90                             | 0.563                                       | 0.884                                 |
| 2     | <b>1.257</b>                     | 0.874                                       | 1.863                                 |
| 3     | 1.727                            | 1.236                                       | -                                     |
| 4     | 2.086                            | 1.456                                       | -                                     |
| 5     | 2.473                            | -                                           | -                                     |
| 6     | 3.371                            | -                                           | -                                     |
| 7     | 3.812                            | -                                           | -                                     |
| 8     | 4.694                            | -                                           | -                                     |
| 9     | 6.300                            | -                                           | -                                     |



**Figure 4: HPLC chromatogram of Mesuaferrone A**



**Figure 5: HPLC chromatogram of stamens of *Mesua ferrea* L., unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb.**

### High-Resolution Orbitrap Liquid Chromatograph Mass Spectrometer (HR-LCMS-Orbitrap)

The HR-LCMS-Orbitrap analysis of the stamens of *Mesua ferrea* L. (table 2) revealed a rich array of unique secondary metabolites, including flavonoids, phenolic acids, and terpenoids, which collectively account for its broad therapeutic potential. In contrast, the substitutes used as adulterants lack the complete medicinal profile and the specific bioactive compounds characteristic of *Mesua ferrea* L. *Cinnamomum tamala* T. Nees & Eberm. (table 3) contains trigonelline and arecoline, the latter of which carries potential carcinogenic risks, while *Dillenia pentagyna* Roxb. (table 4) offers beneficial compounds like quercetin and trigonelline but

lacks critical xanthenes and distinctive flavonoids that are central to the therapeutic efficacy of *Mesua ferrea* L. Figures 6, 7, and 8 illustrate the LC/MS chromatograms of *Mesua ferrea* L., *Cinnamomum tamala* T. Nees & Eberm., and *Dillenia pentagyna* Roxb., respectively. The phytochemical composition of *Mesua ferrea* L. includes unique bioactive compounds such as 4-hydroxycoumarin, naringenin, hesperetin, genistein, catechin, fisetin, isochamaejasmin, isoliquiritigenin, betulin, 18- $\beta$ -glycyrrhetic acid, schweinfurthin B, 5,7-dihydroxy-2-(3-hydroxy-4-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxy} phenyl)-4H-chromen-4-one, vitexin, and pelargonidin. These distinctive metabolites, absent in the adulterants, can be utilized as reliable biomarkers to verify the authenticity of *Mesua ferrea* L.

**Table 2: Identification of compounds in methanol extract of *Mesua ferrea* L. stamens using their retention times, LC/MS and LC-MS/MS data.**

| Sr. No. | R. time | Compounds                                                                                                                          | Molecular formula                               | MS         | Mode (+/-) | MS/MS                      |
|---------|---------|------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|------------|------------|----------------------------|
| 1       | 1.34    | Tropine                                                                                                                            | C <sub>8</sub> H <sub>15</sub> NO               | 142        | +          | 124                        |
| 2       | 1.70    | DL-Stachydrine                                                                                                                     | C <sub>7</sub> H <sub>13</sub> NO <sub>2</sub>  | 144        | +          | 126, 98                    |
| 3       | 6.85    | Trigonelline                                                                                                                       | C <sub>7</sub> H <sub>7</sub> NO <sub>2</sub>   | 138        | +          | <b>120, 92, 77</b>         |
| 5       | 7.9     | Indole-3-acetic acid                                                                                                               | C <sub>10</sub> H <sub>9</sub> NO <sub>2</sub>  | <b>176</b> | +          | 130, 103, 115, 118         |
| 6       | 8.72    | Catechin                                                                                                                           | C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>  | 291        | -          | 245, 229, <b>183</b> , 169 |
| 7       | 9.27    | Jasmonic acid                                                                                                                      | C <sub>12</sub> H <sub>18</sub> O <sub>3</sub>  | 211        | +          | 193, 175, 147, 119         |
| 8       | 9.52    | Kuromanin                                                                                                                          | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 301        | +          | 255, 119                   |
| 9       | 9.70    | Orientin                                                                                                                           | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 431        | +          | 269, 157                   |
| 10      | 9.92    | Quercetin                                                                                                                          | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>  | 301        | +          | 179, 151, 125              |
| 11      | 9.92    | Quercetin-3 $\beta$ -D-glucoside                                                                                                   | C <sub>21</sub> H <sub>20</sub> O <sub>12</sub> | 463        | +          | 301, 179, 151              |
| 12      | 10.12   | Vitexin                                                                                                                            | C <sub>21</sub> H <sub>20</sub> O <sub>10</sub> | 431        | +          | 269, 157                   |
| 13      | 10.127  | 5-Methoxysalicylic acid                                                                                                            | C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>    | 197        | -          | 169, 137, 107              |
| 14      | 10.26   | Robinetin                                                                                                                          | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>  | 301        | +          | 179, 151                   |
| 15      | 10.34   | Trifolin                                                                                                                           | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 303        | +          | 179, 151                   |
| 16      | 10.75   | Cyanidin                                                                                                                           | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>  | 287        | +          | 257, 179                   |
| 17      | 10.83   | Morin                                                                                                                              | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>  | 301        | +          | 151, 125                   |
| 18      | 10.84   | 5,7-dihydroxy-2-(3-hydroxy-4-<br>{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy<br>6-(hydroxymethyl)oxan-<br>2yl]oxy}phenyl) 4 Hchromen-4-one | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 435        | +          | 271, 179                   |
| 19      | 10.96   | 4-Hydroxycoumarin                                                                                                                  | C <sub>9</sub> H <sub>6</sub> O <sub>3</sub>    | 161        | +          | 117, 103                   |
| 20      | 12.35   | Kaempferol                                                                                                                         | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>  | 285        | +          | 241, 151                   |
| 21      | 12.353  | Luteolin                                                                                                                           | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>  | 285        | -          | 151, 179                   |
| 22      | 12.378  | Quercetin                                                                                                                          | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>  | 301        | -          | 179, 151                   |
| 23      | 12.59   | Naringenin                                                                                                                         | C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>  | 271        | +          | 151, 119                   |
| 24      | 12.98   | Hesperetin                                                                                                                         | C <sub>16</sub> H <sub>14</sub> O <sub>6</sub>  | 301        | +          | 151, 179                   |
| 25      | 13.25   | Pelargonidin                                                                                                                       | C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>  | 287        | +          | 179, 151                   |
| 26      | 13.25   | Genistein                                                                                                                          | C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>  | 271        | +          | 135, 119                   |
| 27      | 13.3    | Apigenin                                                                                                                           | C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>  | 269        | -          | 151, 119                   |
| 28      | 13.41   | Fisetin                                                                                                                            | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>  | 285        | +          | 179, 151                   |
| 29      | 13.45   | Isochamaejasmin                                                                                                                    | C <sub>30</sub> H <sub>22</sub> O <sub>10</sub> | 543        | +          | 528, 273                   |
| 30      | 14.30   | Isoliquiritigenin                                                                                                                  | C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>  | 255        | +          | 179, 151                   |
| 31      | 18.53   | Nootkatone                                                                                                                         | C <sub>15</sub> H <sub>22</sub> O               | 221        | +          | 121, 95                    |
| 32      | 21.27   | Betulin                                                                                                                            | C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>  | 441        | +          | 426, 411                   |
| 33      | 22.97   | 18- $\beta$ -Glycyrrhetic acid                                                                                                     | C <sub>30</sub> H <sub>46</sub> O <sub>4</sub>  | 471        | +          | 455, 439                   |
| 34      | 24.09   | Schweinfurthin B                                                                                                                   | C <sub>35</sub> H <sub>46</sub> O <sub>6</sub>  | 421        | +          | 392, 307                   |

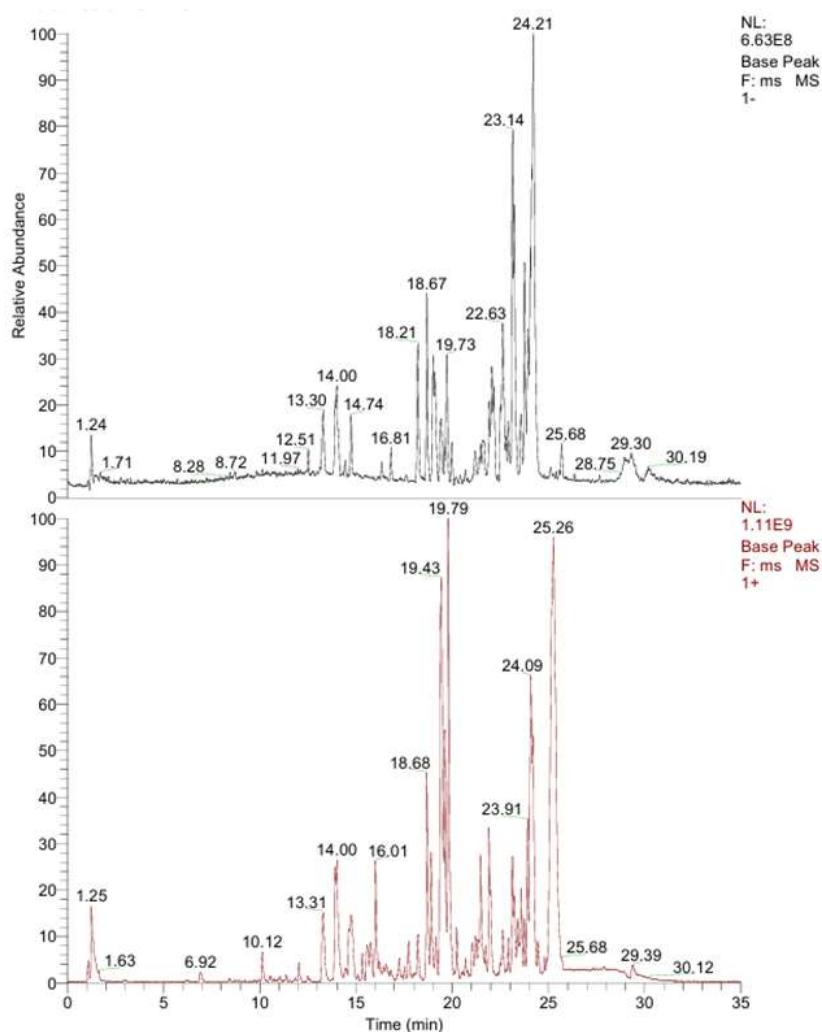


Figure 6: LC/MS chromatogram of *Mesua ferrea* L. stamens.

Table 3: Identification of compounds in methanol extract of *Cinnamomum tamala* T. Nees & Eberm. unripe fruits using their retention times, LC/MS and LC–MS/MS data.

| Sr. No. | R. time | Compounds                                        | Molecular formula                                | MS  | Mode (+/–) | MS/MS              |
|---------|---------|--------------------------------------------------|--------------------------------------------------|-----|------------|--------------------|
| 1       | 1.256   | Trigonelline                                     | C <sub>7</sub> H <sub>7</sub> N O <sub>2</sub>   | 137 | +          | 108, 94, 82, 70    |
| 2       | 1.292   | DL-Stachydrine                                   | C <sub>7</sub> H <sub>13</sub> N O <sub>2</sub>  | 204 | +          | 186, 158, 144, 117 |
| 3       | 1.326   | Arecoline                                        | C <sub>8</sub> H <sub>13</sub> N O <sub>2</sub>  | 97  | +          | 82, 69, 56         |
| 4       | 16.539  | 4-methoxy-6-(prop-2-en-1-yl)-2H-1,3-benzodioxole | C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>   | 137 | +          | 122, 107, 92       |
| 5       | 17.758  | Safingol                                         | C <sub>18</sub> H <sub>39</sub> N O <sub>2</sub> | 320 | +          | 292, 262, 234, 180 |
| 6       | 18.141  | D-Sphingosine                                    | C <sub>18</sub> H <sub>37</sub> N O <sub>2</sub> | 298 | +          | 280, 264, 246, 230 |

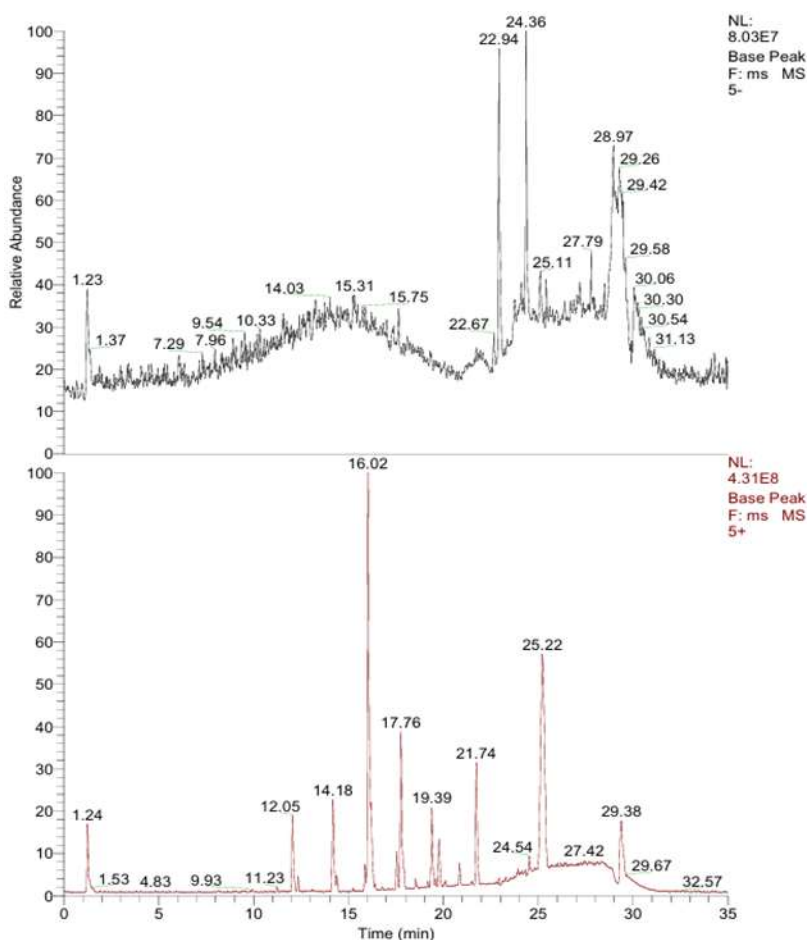


Figure 7: LC/MS chromatogram of *Cinnamomum tamala* T. Nees & Eberm. unripe fruits.

Table 4: Identification of compounds in methanol extract of *Dillenia pentagyna* Roxb. fruits using their retention times, LC/MS and LC-MS/MS data.

| Sr. No. | R. time | Compounds                                                                                                               | Molecular formula                               | MS  | Mode (+/-) | MS/MS              |
|---------|---------|-------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|-----|------------|--------------------|
| 1       | 1.274   | D-(-)-Quinic acid -                                                                                                     | C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>   | 191 | -          | 173, 129, 111, 85  |
| 2       | 1.304   | DL-Stachydrine                                                                                                          | C <sub>7</sub> H <sub>13</sub> N O <sub>2</sub> | 142 | +          | 124, 96, 70, 55    |
| 3       | 1.318   | Trigonelline                                                                                                            | C <sub>7</sub> H <sub>7</sub> N O <sub>2</sub>  | 138 | +          | 123, 110, 95, 84   |
| 4       | 10.832  | Quercetin                                                                                                               | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>  | 302 | +          | 273, 255, 153, 137 |
| 5       | 10.832  | 2-(3,4 dihydroxy phenyl)-5,7-dihydroxy-3- {[ (2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy }-4H-chromen-4-one | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 464 | +          | 447, 302, 285, 153 |
| 6       | 13.343  | (5E)-7-methylidene-10-oxo-4-(propan-2-yl)undec-5-enoic acid                                                             | C <sub>15</sub> H <sub>24</sub> O <sub>3</sub>  | 252 | +          | 237, 223, 209, 191 |
| 7       | 19.891  | Nootkatone                                                                                                              | C <sub>15</sub> H <sub>22</sub> O               | 218 | +          | 203, 189, 175, 161 |
| 8       | 22.958  | Oleamide                                                                                                                | C <sub>18</sub> H <sub>35</sub> N O             | 282 | +          | 264, 222, 204, 180 |

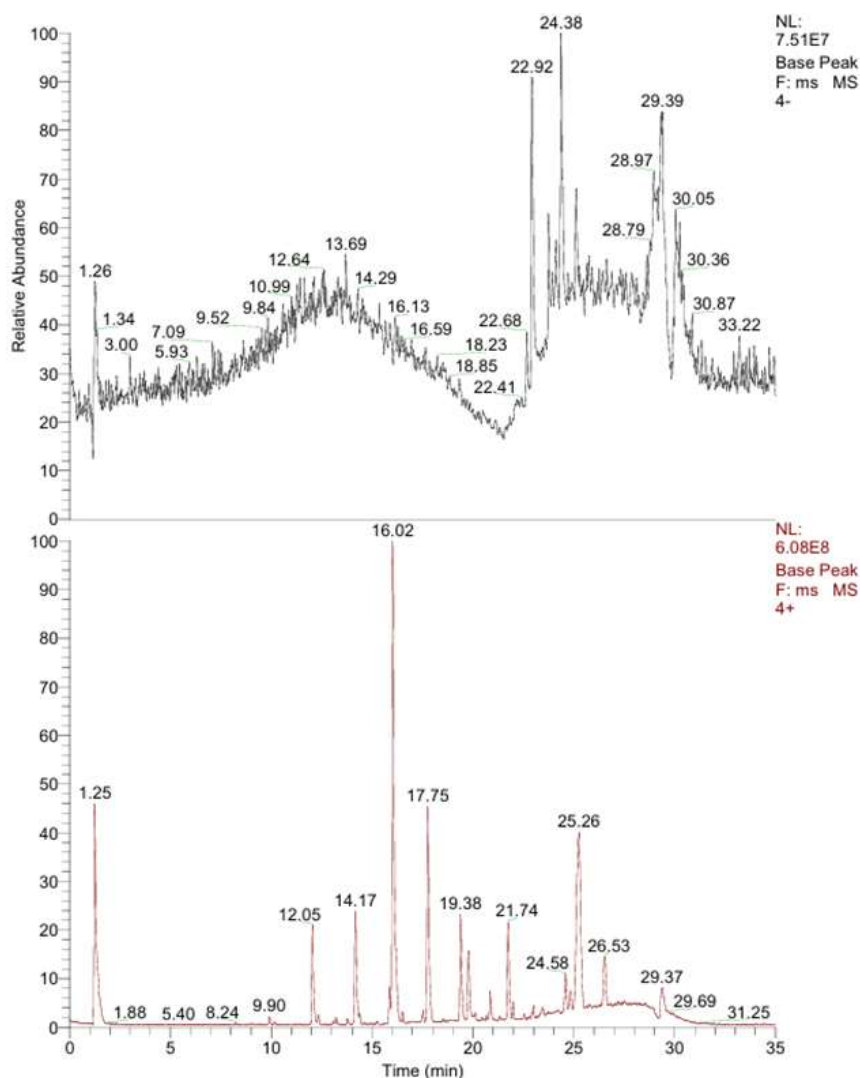


Figure 8: LC/MS chromatogram of *Dillenia pentagyna* Roxb. fruits

## DISCUSSION

According to the National Medicinal Plant Board, Ministry of AYUSH, Government of India, New Delhi, the annual domestic demand for *Mesua ferrea* L. stamens is approximately 150 metric tons, underscoring their vital importance in traditional medicine [23]. However, their high cost, seasonal availability, and labour-intensive collection process make them rare and expensive. This has led to the adulteration of *Mesua ferrea* L. stamens with more readily available and cost-effective substitutes such as the unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. [24]. Despite having no

morphological resemblance to *Mesua ferrea* L., these adulterants are used primarily due to economic factors and their ease of availability. Furthermore, this practice might also stem from unawareness or a lack of knowledge about the authentic source of Nagkesar, leading to inadvertent substitution [25]. The purpose of this study was to scientifically evaluate whether unripe fruits of *Cinnamomum tamala* T. Nees & Eberm. and fruits of *Dillenia pentagyna* Roxb. offer same chemical profile as stamens of *Mesua ferrea* L.

Advanced analytical techniques, including FEGSEM, HPLC, and HR-LCMS-Orbitrap provided critical insights into the morphological,

chemical, and phytochemical differences among the three plant samples.

The FEGSEM analysis of *Mesua ferrea* L. stamens revealed smooth, oval-shaped pollen grains with a fine reticulate pattern and a tricolpate structure. This unique morphology is crucial for species identification, enabling efficient pollination and protecting the grains. In contrast, *Cinnamomum tamala* T. Nees & Eberm. displayed pollen grains that were spherical with a monocolpate structure and a moderately perforated exine, indicating a different pollination strategy. *Dillenia pentagyna* Roxb. pollen, similar in shape to *Mesua ferrea* L., exhibited a tricolpate structure but with less perforated exine. These differences highlight distinct morphological characteristics that differentiate *Mesua ferrea* L. from its potential adulterants, *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb., underlining the unique chemical profile of *Mesua ferrea* L. However, a more quantitative morphometric analysis would be beneficial in future studies to further substantiate these differences.

The HPLC analysis highlighted considerable differences in the phytochemical profiles of the three samples. The retention times and peak areas of key compounds varied significantly. Mesuaferrone A, a biflavonoid with reported tyrosinase and elastase inhibitory activities [22], was detected exclusively in the stamens of *Mesua ferrea* L., serving as a reliable biomarker for its authenticity. The chromatograms (see Figures 4 and 5 and Table 1) clearly illustrate that the retention times and peak areas of key compounds differ significantly among the samples. Although our study did not include detailed statistical significance testing, the consistency of triplicate analyses supports the reproducibility of these findings.

The detection of Isochamaejasmin via HR-LCMS-Orbitrap, which has a similar molecular weight and formula to Mesuaferrone A, suggests that these compounds may belong to a related class of secondary metabolites. When compared with previous studies, the concentration levels of Mesuaferrone A in *M. ferrea* L. appear consistent with earlier reports [26], [27], although the presence of potential structural analogues like Isochamaejasmin could indicate novel aspects of its phytochemical profile that merit further quantitative investigation.

The HR-LCMS-Orbitrap analysis also revealed a rich array of secondary metabolites, including flavonoids, xanthenes, and terpenoids such as catechin, quercetin, naringenin, and isoliquiritigenin—compounds that are crucial to the therapeutic properties of *Mesua ferrea* L. In contrast, *Cinnamomum tamala* T. Nees & Eberm. unripe fruits were found to contain arecoline, compound linked to potential health risks if consumed in excess. *Dillenia pentagyna* Roxb. fruits also contained quercetin and trigonelline, but notably lacked the xanthenes and specific flavonoids that are essential to the medicinal efficacy of *Mesua ferrea* L.

These findings not only align with but also extend previous research by providing detailed mass spectrometric data that highlight the distinct chemical signatures of these botanicals. Although the current study's quantitative analysis was primarily qualitative in nature, the reproducibility of the chromatographic and mass spectrometric data across triplicate analyses lends credence to the observed differences.

Despite these insights, the study has several limitations. The absence of rigorous statistical interpretation means that the statistical significance of the differences observed could not be formally established. Furthermore, potential



sources of error—such as sample heterogeneity, variations in extraction efficiency, and instrumental variability—could influence the results. Future research should incorporate more extensive quantitative analyses, including appropriate statistical tests and larger sample sizes, to further validate these findings.

The broader implications of these results are significant. The distinct chemical and morphological profiles of *Mesua ferrea* L. stamens underscore the risks associated with adulteration, as the substitutes do not offer comparable therapeutic benefits. Ensuring the authenticity of botanical ingredients is critical not only for the efficacy of Ayurvedic formulations but also for applications in pharmacology, food science, and natural product research. The unique bioactive profile of *Mesua ferrea* L. could serve as a basis for developing standardized herbal extracts with defined therapeutic properties, while the detailed analytical techniques employed here could be adapted for quality control in other industries.

The substitution of medicinal plants is a common practice, but it is important to ensure that the substitutes provide equivalent therapeutic benefits [28]. Such practices compromise the quality and efficacy of traditional medicine, as the adulterants lack the distinct bioactive compounds and therapeutic properties found in *Mesua ferrea* L. stamens. The study revealed that while *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. are traditionally and scientifically known for their medicinal properties, their fruits did not offer any therapeutic benefits equivalent to *Mesua ferrea* L. stamens. This is due to significant differences in their bioactive compounds, pharmacological effects, and sensory properties [29]. Comparative studies on these aspects can provide deeper insights into the validity and impact of such substitutions.

## CONCLUSION

This study demonstrates that while *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb. have recognized medicinal properties, their fruits do not offer the same therapeutic benefits as *Mesua ferrea* L. stamens. Advanced analytical methods such as FEGSEM, HPLC, and HR-LCMS-Orbitrap have revealed significant differences in their morphological, chemical, and phytochemical properties. FEGSEM analysis indicated that *Mesua ferrea* L. stamens possess smooth, oval-shaped pollen grains with a fine reticulate pattern and a tricolpate structure, which are not replicated in the pollen grains of *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb., which are predominantly monocolpate with less perforated exine. Chromatographic analysis showed that *Mesua ferrea* L. stamens contain Mesuaferrone A, a specific biflavonoid, which was completely absent in both *Cinnamomum tamala* T. Nees & Eberm. and *Dillenia pentagyna* Roxb.

These findings advance our understanding of the chemical uniqueness of *Mesua ferrea* L. and underscore the importance of employing rigorous analytical methods in validating the authenticity of medicinal plants used in traditional formulations. The study contributes to the field by providing a detailed comparative analysis that highlights the risks associated with botanical adulteration and emphasizes the need for stringent quality control in herbal medicine.

However, the study is not without limitations. The absence of robust statistical validation and the relatively limited sample set may affect the generalizability of the findings. Future research should focus on expanding the sample size, integrating quantitative statistical analyses, and exploring additional bioactive compounds through in vitro and in vivo pharmacological studies. These



steps will further elucidate the therapeutic potential of *Mesua ferrea* L. and refine the strategies used to distinguish it from potential substitutes, thereby enhancing the quality and efficacy of traditional and commercial products.

## GLOSSARY:

FEGSEM: Field Emission Gun Scanning Electron Microscopy; HPLC: High-Performance Liquid Chromatography, HR-LCMS-Orbitrap: High-Resolution Orbitrap Liquid Chromatograph Mass Spectrometer (HR-LCMS-Orbitrap)

## CONFLICT OF INTEREST:

The authors declare no conflicts of interest.

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