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FTIR FINGERPRINTING FOR CHEMOTAXONOMIC AUTHENTICATION OF *TECOMELLA UNDULATA* STEM BARK

V. Nagaraju, *M. Mahendranath, K. Madhava chetty
Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh
**Author for Correspondence : mitta.mahendranath@gmail.com*

ABSTRACT
Tecomella undulata (Sm.) Seem., traditionally known as Rohitaka, is a pharmacologically significant medicinal plant in Ayurvedic medicine, valued for its antibacterial, analgesic, hepatoprotective, and anti-HIV properties. Due to its high therapeutic demand, the market often faces challenges with botanical substitution and adulteration. This study aims to establish a simplified Fourier Transform Infrared (FTIR) spectroscopy fingerprint to facilitate the chemotaxonomic authentication of *T. undulata* stem bark and distinguish it from common botanical adulterants used in the trade of Rohitaka. Methanolic extracts of the stem bark were prepared via maceration and analyzed to establish a comparative FTIR baseline. The resulting spectral profiles provide a definitive chemical signature, enabling the verification of authentic plant samples against market substitutes. These findings establish FTIR fingerprinting as a robust, reproducible, and critical quality control parameter for the herbal industry, ensuring the phytochemical integrity of *Tecomella undulata* in medicinal formulations.

Keywords: *Tecomella undulata, Rohitaka, FTIR fingerprinting, chemotaxonomic authentication.*

INTRODUCTION
Indian medicinal plants constitute the foundation of a global phytotherapeutic legacy, originating from ancient systems like Ayurveda, Siddha, and Unani. These botanical resources serve as a vital nexus, translating indigenous knowledge into modern integrative healthcare solutions. Integrating Ayurvedic, Siddha, Unani principles with contemporary ethnobotanical and pharmacological research has significantly advanced the identification of potent bioactive compounds. (Satpati, 2026; Raju & Das, 2024; Tandon & Yadav, 2017; Pandey *et al*., 2013; Bhatt, 2001; Jain, 1981). By bridging tradition with innovation, India's floral diversity provides a scalable and sustainable blueprint for global health security and resilient therapeutic systems (Mathe & Khan, 2026; Raju & Das, 2024; Banu *et al*.,2024; Kumari *et al*.,2023; Khanum, 2022).

However, a critical gap persists between traditional wisdom and modern medical standards, necessitating rigorous scientific and clinical validation to bridge this divide, priority must be given to the standardization of drugs, formulations, comprehensive biochemical profiling, and the urgent conservation of medicinal species threatened by over-exploitation and climate change. (Sethi *et al.*.2025; PMC, 2025; Semwal *et al.,*2019; Sharma *et al.,*2022; Singh & Kumar, 2021; Khare 2007; Kirtikar & Basu,1984,1980).

Integration of chemotaxonomy (classification of organisms based on their chemical constituents) is the bridge between traditional botanical identification and modern pharmaceutical standardization. (Frigerio *et al.,*2024). Rigorous quality control and standardization protocols are indispensable for botanical identification and authentication of herbal drugs, particularly as the majority of industrial and community-sourced materials are harvested from the wild and frequently subjected to adulteration (Gajarmal *et al*.,2026; Alyas *et al.,*2024; Ichim 2019; Shaheen *et al.,*2019). This increasing demand for botanicals serves as the primary driver for fraudulent practices, which not only undermines the credibility and global promotion of herbal medicine but also triggers significant adverse clinical events. (WHO,2025; Shanmughanandhan *et al.,*2016). Furthermore, the sophistication of modern, economically motivated adulteration requires advanced microscopic and chemical analyses, as commercial suppliers utilize increasingly complex scientific processes to bypass conventional detection methods. (Ratnasekhar *et al.,* 2025; Asiamah, *et al.,2025*; Sharma *et al*., 2022; Ichim *et al.,* 2020 & 2019; Chugh *et al.,* 2018; Subramanyam *et al.,* 2016; Black *et al.,*2016; Prakash *et al.,*2013).

About FTIR

Fourier Transform Infrared (FTIR) spectroscopy serves as a high-throughput chemotaxonomic tool, utilizing metabolic fingerprinting to differentiate functional group vibrations unique *to original authentic sample* from its adulterants (Abed *et al*., 2025; Faturachman *et al*., 2025; Chen *et al.,*2023 & 2018; Feng *et al*., 2024)

About Ayurvedic Drug "Rohitaka"

Ayurvedic drug "Rohitaka" (*Tecomella undulata*), tracing its origins in classical Sanskrit texts to its systematic categorization and application in contemporary clinical practice (Thakur *et al.,* 2020). Rohitaka formulations are frequently compromised by taxonomic ambiguities, misidentification during bulk collection, and a pervasive lack of botanical expertise among commercial suppliers, often resulting in the substitution of the genuine bark with chemically non-equivalent or potentially toxic related species. (Nagaraju *et al*., 2020; Thakur *et al.,* 2020; Sharma *et al.,*2002)

Tecomella undulata (Sm.) Seem. (Bignoniaceae) is a deciduous tree locally recognized as Rohitaka or Rakta-Rohida, a significant medicinal species indigenous to India's arid and semi-arid regions (Kumar *et al.* 2026; Keshari *et al.,* 2018 and Sharma 2002). This tree taxon is recognized as Endangered (Vats *et al*., 2025; Arshad *et al*., 2022; Robinson *et al.,*2005) and threatened species (Ghafouri *et al*.,2025).While various *In-vitro* (Poria *et al*.,2017; Robinson *et al.,*2005) and Phenological studies (Daneva *et al*., 2024 & Singh *et al*., 2014) suggest that this species can thrive under Indian environmental conditions and its regenerative capacity is a conflict which remains a point of debate in contemporary research.

Significant medicinal plant in pharmacological research validated and well established (Nagaraju, 2020). Considering the potent medicinal applications and therapeutic integrity of *T. undulata* there is an increasingly compromised by the prevalence of commercial botanical adulterants, such as *Aphanamixis polystachya*, *Ventilago maderaspatana*, *Diospyros ferra* var. *buxifolia*, *Polygonum glabrum* which is commonly available in the market of South India (Nagaraj 2020; Keshari & Bhat 2019; Keshari *et al*.,2018).

The present study aims to establish a robust and efficient analytical framework for the authentication and scientific validation of *T. undulata* stem bark along with a comparative study of its commercial adulterants using Fourier Transform Infrared (FTIR) spectroscopy fingerprinting.

MATERIALS AND METHODS

Plant samples & Authentication

The samples of fresh barks of *Tecomella undulata* (Table 1; Figure 1 A-D) were collected from SVU Campus, Tirupati, Andhra Pradesh (Figure 1&2. A-D). The botanical identification of the taxa was carried out by using regional and local floras (Pullaiah, 2018; Gamble 1918; and Chetty *et al.,* 2019). The bark specimens were collected from the field and local market and deposited in the Herbarium of Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh (Table. 1). Healthy bark was chopped and fixed in the field immediately in FAA (Formalin - 5ml + Acetic acid – 5ml + 70% ethyl alcohol – 90ml) (Nagaraju *et al*., 2020; Ramesh *et al.,* 2013,2014; Sivaji *et al*., 2012). The Plant specimens were authenticated by comparing voucher herbarium specimens with relevant herbaria (K, NYBG,MH, SVUTY) (Table 1). Photographs of authentic taxon *T.undulata* habitat, flowering stage were taken (Figure 1 &2).

Table 1. Details of Plants used in markets including original and adultured taxa of Rohitaka

S. No.	Adulterant taxa	Habitat	Family	Voucher specimen
1.	*Tecomella undulata*	Tree	Bignoniaceae	SVUTY/ BIG/ 4899
2.	*Aphanamixis polystachya*	Tree	Meliaceae	SVUTY/MEL-4082
3.	*Ventilago maderaspatana*	Liana	Rhamnaceae	SVUTY/RHM-4766
4.	*Diospyros ferra* var. *buxifolia*	Tree	Ebenacea	SVUTY/EBE-4143
5.	*Polygonum glabrum*	Herb	Polygonaceae	SVUTY/POL-4419

Figure. 1. *Tecomella undulata* A) Habit B) Author Collection C) Author with specimen D) Flowering twig

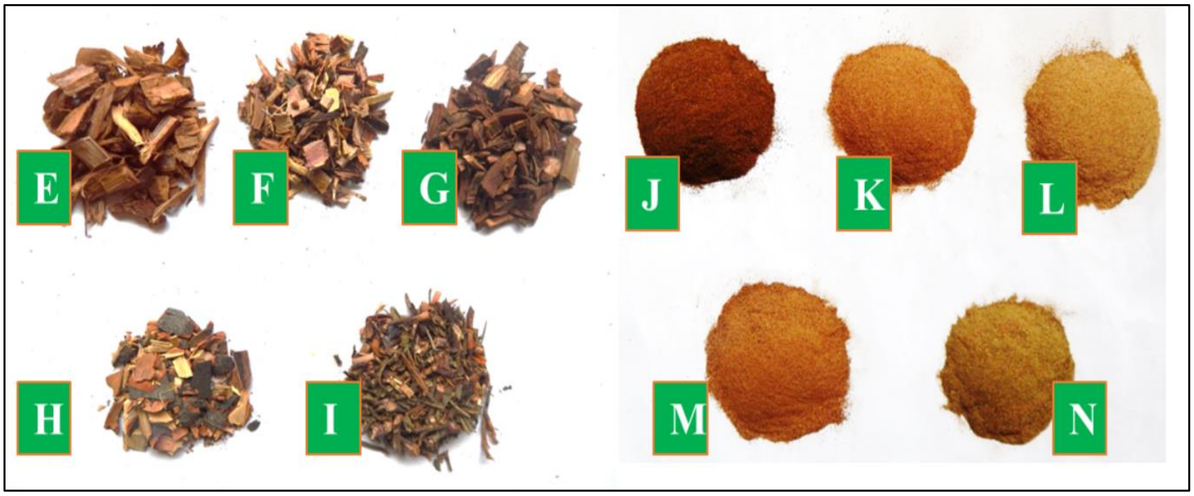


Figure. 2. Dried chopped Bark and its Powder form.
E, J) *Tecomella undulata*; F, K) *Aphanamixis polystachya*; G, L) *Ventilago maderaspatana*; H, M) *Diospyros ferra* var. *buxifolia*, I, N) *Polygonum glabrum*

FTIR studies
The methanolic extracted bark samples listed above (Table.1) was prepared by macerating the bark powder in analytical-grade methanol (1:10 w/v) for 72 hours with periodic agitation. The filtrate was subsequently concentrated under reduced pressure at 40°C using a rotary evaporator to obtain the crude extract, which was stored at 4°C for FTIR Studies and for further pharmacological validation. Chemometric characterization was conducted *via* the standard KBr pellet technique on a PerkinElmer FTIR instrument, scanning across a spectral range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. (Faturachman *et al.,* 2025; Feng *et al*., 2024; Chen *et al.,* 2023 & 2018)

RESULTS AND DISCUSION

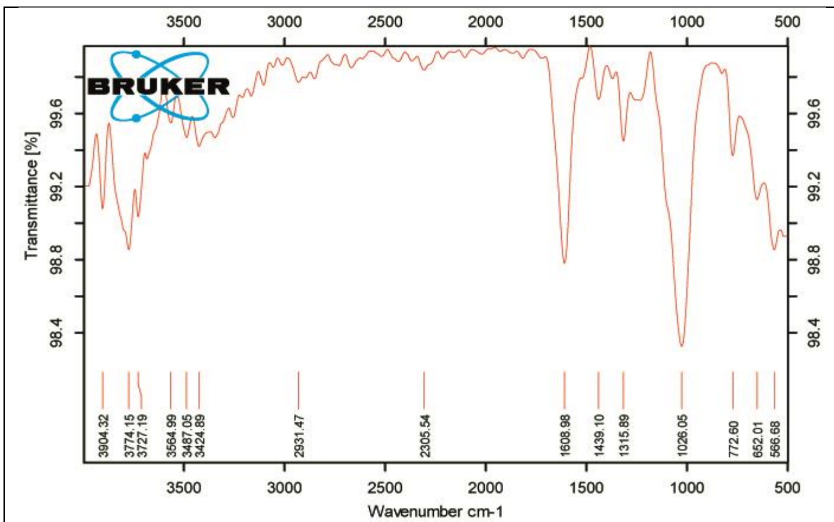
FTIR spectroscopic analysis

FTIR studies of Rohitaka versus its Adulterants
Methanolic extracts of authenticated stem bark samples were prepared via cold maceration Figure 2 represents Dried, chopped Bark and its Powder form of Rohitaka and its adulterants Raw form. These extracts were subjected to Fourier Transform Infrared spectroscopy (FTIR) analysis to delineate a comprehensive profile of functional groups characteristic of the original species (*Tecomella undulata*) against its adulterants (*Aphanamixis polystachya*, *Ventilago maderaspatana*, *Diospyros ferra* var. *buxifolia*, *Polygonum glabrum*).
This research identifies species-specific functional group profiles that serve as distinctive chemotaxonomic markers obtained in the region 4000–650 cm^{-1}. The assignment of functional groups responsible for IR absorption are tabulated and mapped along with the FTIR Spectra (Fig. 3- 7).
FTIR spectra of *T. undulata* exhibited distinct diagnostic variations compared to its common adulterants particularly within the 3900–3200 cm^{-1} hydroxyl region and the 1500–500 cm^{-1} fingerprint region.

Although *T. undulata* and its adulterants share primary phytochemical classes specifically flavonoids, phenolics, glycosides, and naphthoquinones the *T. undulata* samples displayed significantly higher absorption intensities between 3890 and 3300 cm $^{-1}$. Furthermore, prominent peaks at 1605 cm^{-1} (N-H bending), 1317 cm^{-1} (N-O symmetric stretch), 1022 cm^{-1} (C-N stretch), and 766 cm^{-1} (N-H) suggest a higher concentration of nitrogenous constituents, such as alkaloids and amino acids, in *T. undulata* relative to the weaker signals observed in the adulterant profiles (Table 2).

Table 2: FTIR Comparative Analysis: *Tecomella undulata* vs. Adulterant taxa

Plant Species	Original sp./ Adulterant sp.	Unique Functional Groups / Characteristics
Tecomella undulata	Original Rohitaka	C-H stretch of aromatics (uniquely identified at two distinct peaks), C-N stretch of aliphatic amines, and C-Br stretch of alkyl halides.
Aphanamixis polystachya	Adulterant	C-H stretch of alkanes, C-N stretch of aliphatic amines, and C-Cl stretch of alkyl halides.
Ventilago maderaspatana	Adulterant	O-H stretch of alcohols/phenols, C-H stretch of alkanes, and specific C-Br stretch
Diospyros ferra	Adulterant	O-H stretch of alcohols/phenols, C-H stretch of alkanes, and N-H primary/secondary amines.
Polygonum glabrum	Adulterant	Dual C-N stretch of aliphatic amines and unique C-Br stretch at 570.58
Soymida febrifuga	Adulterant	O-H stretch of alcohols/phenols and C-N stretch of aromatic amines.



- Peaks at 3601.21, 3437.03, 3342.91, 3226.19 (**O-H stretch of alcohols/phenols**).
- 3155.12, 3033.21 (**C-H stretch of aromatics**).
- 1605.82 (**N-H bend of primary amines**).
- 1439.02 (**C-C stretch of aromatics**).
- 1317.15 (**N-O symmetric stretch of nitro compounds**).
- 1022.49 (**C-N stretch of aliphatic amines**).
- 766.77 (**N-H primary, secondary amines**).
- 647.28 & 594.76 (**C-Br stretch of alkyl halides**)

Figure 3: FTIR Spectra of *Tecomella undulata* Stem bark powder

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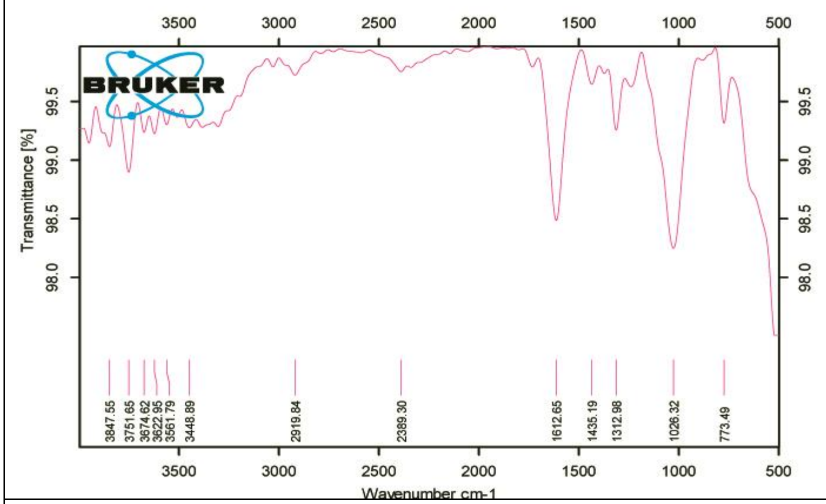
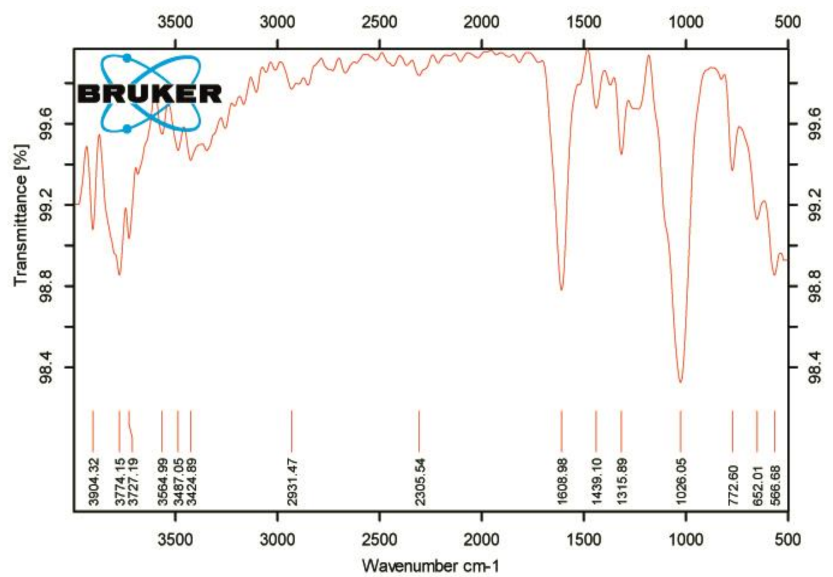


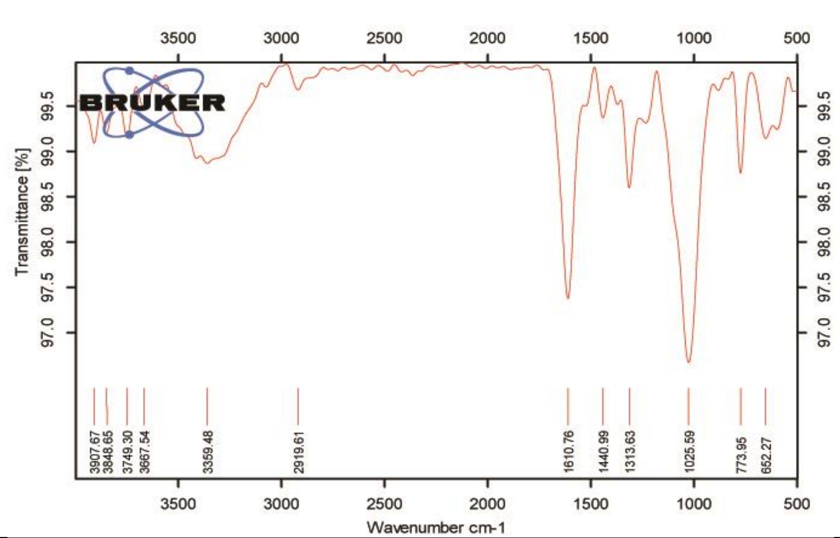
- Peak at 3622.95 **(O-H stretch of alcohols/phenols).**
- 2919.84 **(C-H stretch of alkanes).**
- 1612.65 **(N-H bend of primary amines).**
- 1435.19 **(C-C stretch of aromatics).**
- 1312.98 **(N-O symmetric stretch of nitro compounds).**
- 1026.32 **(C-N stretch of aliphatic amines).**
- 773.49- **(C-Cl stretch of alkyl halides).**

Figure 4: FTIR Spectra of *Aphanamixis polystachya* Stem bark powder



- 3487.05, 3424.89 **(O-H stretch of alcohols/phenols)**
- 2931.47 **(C-H stretch of alkanes)**
- 1608.98 **(N-H bend of primary amines)**
- 1439.10 **(C-C stretch of aromatics)**
- 1315.89 **(N-O symmetric stretch of nitro compounds)**
- 1026.05 **(C-N stretch of aliphatic amines)**
- 772.60 **(N-H primary, secondary amines)**
- 652.01 **(C-Br stretch of alkyl halides).**

Figure 5: FTIR Spectra of *Ventilago maderaspatana* Stem bark powder

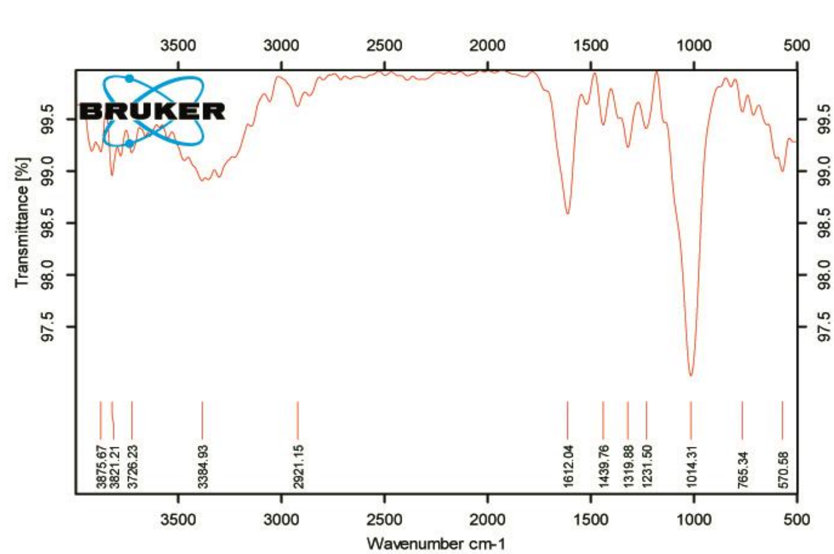


- 3359.48 **(O-H stretch of alcohols/phenols)**
- 2919.61 **(C-H stretch of alkanes)**
- 1610.76 **(N-H bend of primary amines)**
- 1440.99 **(C-C stretch of aromatics)**
- 1313.63 **(N-O symmetric stretch of nitro compounds)**
- 1025.59 **(C-N stretch of aliphatic amines)**
- 773.95 (N-H primary, secondary amines)
- 652.27 **(C-Br stretch of alkyl halides)**

Figure 6: FTIR analysis of *Polygonum glabrum* – Aerial plant parts powder

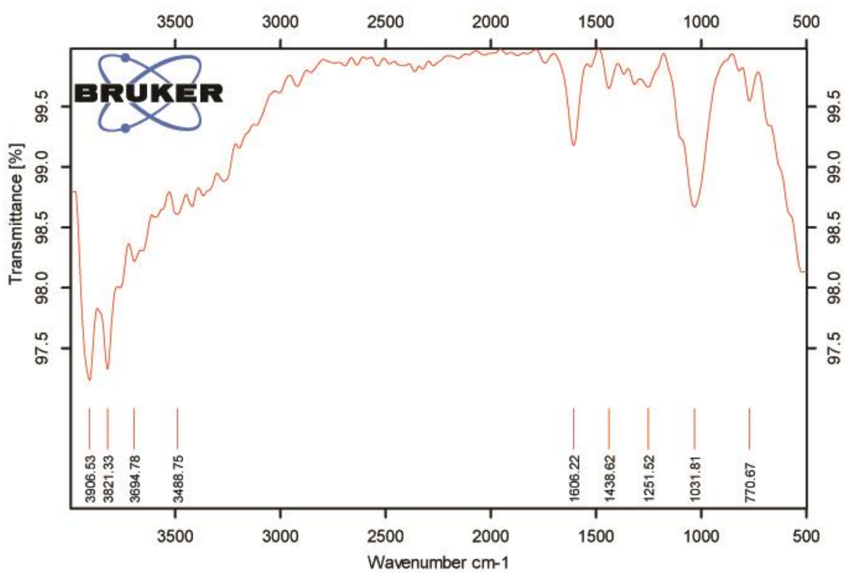
Chemosystematic validation & Regulatory framework for Tecomella undulata

FTIR spectroscopy allows the mapping of functional groups (aliphatic/aromatic alcohols, esters, ethers) that act as chemical fingerprints to distinguish *T. undulata* from its congeneric allies. *T. undulata* considered as endangered status, this study aligns with WHO strategies promoting sustainable sourcing and the use of micropropagation to protect natural biodiversity while meeting industrial demand. as the first documented effort to utilize FTIR fingerprinting specifically for the stem bark of *T. undulata,* this research offers a rapid, non-destructive, and cost-effective technique for quality control (Srirama *et al.,*2017). These findings establish a vital benchmark for the standardization and regulatory validation of *Tecoma undulata* (Rohitaka) based phytotherapeutics. Authenticating *Tecomella undulata* ensures that the final pharmaceutical formulation remains a safe and effective medicine, upholding the global standards for traditional drug integration.



- 3384.93 **(O-H stretch of alcohols/phenols)**
- 2921.15 **(C-H stretch of alkanes)**
- 1612.04 **(N-H bend of primary amines)**
- 1439.76 **(C-C stretch of aromatics)**
- 1319.88 **(N-O symmetric stretch of nitro compounds)**
- 1231.50, 1014.31 **(C-N stretch of aliphatic amines)**
- 765.34 **(N-H primary, secondary amines)**
- 570.58 **(C-Br stretch of alkyl halides)**

Figure 7: FTIR spectra of *Diospyros ferra* var. *buxifolia* - Stem bark powder



- 3694.78, 3488.75 **(O-H stretch of alcohols/phenols)**
- 1606.22 **(N-H bend of primary amines)**
- 1438.62 **(C-C stretch of aromatics)**
- 1251.52 **(C-N stretch of aromatic amines)**
- 1031.81 **(C-N stretch of aliphatic amines**
- 770.67 **(N-H wag of primary, secondary amines)**

Figure 8: FTIR spectra of *Soymida febrifuga* - Stem bark powder

Digital Resources such as IMPPAT 2.0 (Vivek-Ananth *et al*., 2023), CDMMM by Vinay *et al*. (2026) and DIMPSAR dataset by Pushpa and Rani (2023) adress the critical need for chemotaxonomic studies that link plant metabolite diversity, chemical markers with advanced figerprinting techniques to enhance species recognition and the therapeutic precission in this modern era. Integrating the phylogenetic framework of APG IV with the high-resolution functional group mapping of FTIR, this investigation advances contemporary chemosystematics by validating the unique taxonomic position of *T. undulata* and establishing a rigorous chemical benchmark for industrial standardization. Integrating advanced machine learning-guided metabolomic fingerprinting into the Indian herbal sector is critical for establishing empirical authenticity standards, enabling the precise identification of geographical origin and species purity required to safeguard India's global trade in traditional medicines (Ratnasekhar *et al.,* 2025; Sharma *et al*.,2025; Vinay *et al.,* 2026).

CONCLUSION

This study establishes FTIR spectroscopy as a robust chemotaxonomic tool for the authentication of *Tecomella undulata* (Rohitaka) stem bark. By analyzing methanolic extracts of authenticated samples against common adulterants *Aphanamixis polystachya*, *Ventilago maderaspatana*, *Diospyros ferrea* var. *buxifolia*, and *Polygonum glabrum* which posses unique biochemical signatures were successfully delineated. These distinctive functional group profiles provide a rapid, non-destructive, and cost-effective biochemical fingerprint for precise botanical identification. Consequently, this FTIR spectroscopic framework offers a reliable diagnostic benchmark for the pharmaceutical industry, ensuring raw material purity and facilitating the regulatory validation of *T. undulata* based herbal formulations in the global market.

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