INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
25–333/2025/34:340409
http://www.fspublishers.org

Full Length Article

Determination of Phytochemicals in *Ficus deltoidea*, *Lawsonia inermis* and *Morinda citrofolia* Leaves using Raman Spectroscopy

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Received 05 April 2025; Accepted 21 May 2025; Published online 29 June 2025

Editor: Abdul Wahid

Abstract

The identification of phytochemicals in medicinal plants is crucial for understanding their bioactive compounds and therapeutic properties. Raman spectroscopy is an optical tool that has been recently proposed as one of the most promising, immediate, label-free and non-destructive method of analysis of plant substances. This study determined the phytochemical structures in the leaves of *Ficus deltoidea*, *Lawsonia inermis* and *Morinda citrofolia*, three plants widely known for their medicinal uses utilizing Raman spectroscopy. Plant leaves were subjected to a Raman spectrometer with 785 nm laser to acquire spectral data. The spectra were baseline corrected, and vector normalized following acquisition. Major bands of the leaves showed prominent aromatics, phenylpropanoids, carotenoids and cell wall components. The spectra provided distinct vibrational modes corresponding to Raman bands particularly carotenoids and aromatics that offer insight into the medicinal benefits of plant phytochemicals. The results highlighted the potential of Raman spectroscopy as an efficient tool for the analysis of medicinal plants; thus, enabling faster and more precise identification of phytochemicals. This study contributes to a broader application of Raman spectroscopy in medicinal plant research for their use in agricultural, pharmaceutical and nutraceutical industries.

Keywords: Raman spectroscopy; Phytochemical; *Ficus deltoidea*; *Lawsonia inermis*; *Morinda citrifolia*

Introduction

The use of medicinal plants dates back thousands of years. Over the time, herbal remedies became an integral part of traditional healing systems, like Traditional Chinese Medicine (TCM) that has been practiced in China for thousands of years to maintain health (Liu *et al*. 2025; Xu *et al*. 2025). These systems carefully studied the effects of various plants' active ingredients and developed a comprehensive understanding of their therapeutic benefits. Nowadays, TCM evolved into modern medicine with detailed knowledge of specific herbs for treating specific ailments.

Active ingredients in plants, referred as phytochemicals, are responsible for the therapeutic effects of the medicinal plants. Generally, phytochemicals are compounds from plant secondary metabolites that protect the plant from abiotic or biotic stresses (Shahzad *et al*. 2025; Sultana *et al*. 2025). Phytochemicals include a wide range of compounds like alkaloids, flavonoids, terpenes and polyphenols with specific roles in promoting health or treating illnesses including antioxidant (Wang *et al*. 2025) and analgesic or anti-inflammatory properties (Otu-Boakye *et al*. 2024). At the present day, many phytochemicals were isolated from plants and become the groundwork for modern pharmacology (Pachal *et al*. 2025; Ragab *et al*. 2025).

The phytochemicals are often quantified with analytical chemistry tools such as high-performance liquid chromatography (HPLC). This method requires laborious preparation including extraction of phytochemicals from the tissue that typically needs abundant tissue for the analysis. The extraction methods are highly dependent on factors like polarity, pH and temperature, which the possibility of side reaction and rearrangement of chemical structures may occur (Cetinkaya *et al*. 2025; Kevat *et al*. 2025). Other analytical techniques such as mass spectrometry, infrared spectrometry, and liquid or gas chromatography also require rigorous sample preparation and highly time-consuming (Park *et al*. 2023). To rapidly detect phytochemicals under different intrinsic and extrinsic factors, conventional methods are not reliable. Thus, an alternative instant detection method to determine plant phytochemicals is needed.

To cite this paper: Tantowi NACA (2025). Determination of phytochemicals in *Ficus deltoidea*, *Lawsonia inermis* and *Morinda citrofolia* leaves using raman spectroscopy. *Intl J Agric Biol* 34:340409. https://doi.org/10.17957/IJAB/15.2381

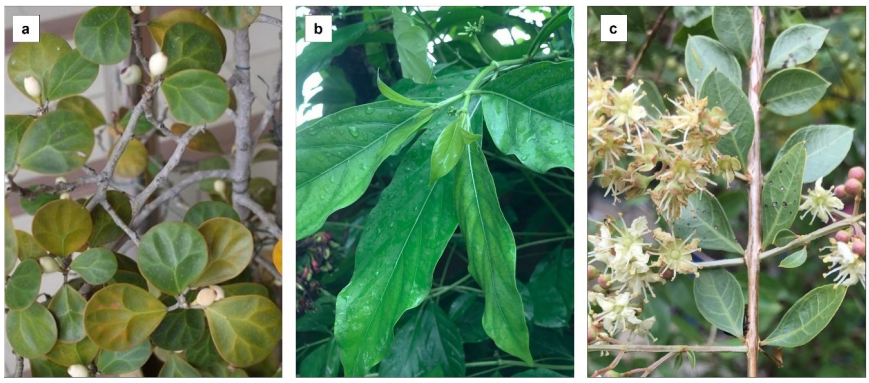

Fig. 1: Medicinal plants. (**a**) *Ficus deltoidea* var. *deltoidea,* (**b**) *Morinda citrifolia* var. *citrifolia* and (**c**) *Lawsonia inermis*

Raman spectroscopy is an analytical tool used to study molecular vibrations, rotations and other low-frequency modes (Huang *et al*. 2025). It relies on the inelastic scattering of light to gather information about the vibrational states of molecules. Basically, when light interacts with a molecule, most of the light is elastically scattered or has the same energy as the incoming light. However, a portion of the light is in-elastically scattered, which means that the energy is different from original incident. This energy difference corresponds to the vibrational energy levels of the molecule and reveal specific biochemical fingerprint of a material.

The sample preparation for Raman spectroscopy is usually minimal as analysis can often be carried out using the whole leaf of tissue and a spectrum acquired within seconds can directly be interpreted with spectral libraries (Pezzotti *et al.* 2024). Further, Raman spectroscopy is suitable for to perform *in situ* or direct with fresh plant materials due to weak Raman scattering for water (Wu *et al*. 2024). Despite the immense potential of the non-destructive rapid spectroscopic technique, there are few reports describing the use of Raman spectroscopy in medicinal plants. The aim of this study was to utilize Raman spectroscopy for direct and rapid identification of phytochemicals present in *Ficus deltoidea, Lawsonia inermis* and *Morinda citrifollia.*

Materials and Methods

Plant materials

Mature leaves of medicinal plants were chosen from healthy growing plants in Kedah, Malaysia (6° 26' 11.9832" N, 100° 26' 58.5024" E). The plants were *Ficus deltoidea* var *deltoidea* (Mistletoe fig), *Morinda citrifolia* var *citrifolia* (Noni) and *Lawsonia inermis* (Henna) (Fig. 1). The leaves were authenticated by agronomist at Universiti Utara Malaysia. Leaves were collected, washed and kept at 4°C until Raman spectra acquisition, occurring within the following 48 h. Leaves were used for this study to mimic the traditional herbal medicine leaves used in concoction, tea or edible herbs. Leaves were cut in 1 × 1 cm pieces at the middle part prior to spectroscopy analysis avoiding midrib and veins.

Raman spectroscopy

Leaf specimens were positioned flat with the adaxial surface of the leaves facing the air on an aluminium plate for Raman spectrometer. Spectra acquisition was performed with minimum laser power and accumulation time to avoid damaging or burning the samples. The leaves were subjected to Renishaw inVia micro-spectrometer (Renishaw plc, Gloucestershire, UK) connected with a 785 nm solid-state laser delivering a power of 0.0001% at the leaf surface. The laser beam was focused through a 50x long working distance objective. The spectra were collected with 10 sec of integration time thrice. Spectra covering the range of 600–1800 cm^{-1} were recorded for each specimen. This procedure was repeated for varying spatial distribution of the leaf.

Data analysis

To analyze only the Raman peaks, it was needed to correct the baseline profile as the measurements included fluorescent spectra as well. After baseline correction, vector normalization was applied by dividing each band of the spectrum by norm value using Raman WIRE software (Renishaw plc, Gloucestershire, UK). The spectra were averaged to produce 1 spectrum for each medicinal plant leaf. The spectra obtained were validated and analysed based on wavelengths of Raman shift and Raman intensity of the peaks of phytochemicals compared to the relevant literatures (Jain *et al*. 2024; Juárez and Kurouski 2024).

Results

Major raman bands and their assignments

Carotenoids: The Raman spectra obtained from leaves including mistletoe fig, henna and noni displayed several prominent peaks, which were assigned to specific phytochemicals (Table 1). Notably, peaks at 1155 cm^{-1} and

Table 1: Vibrational bands and their assignments from medicinal plant leaves; *F. deltoidea* (Mistletoe fig), *L. inermis* (Henna) and *M. citrifolia* (Noni)

Band (cm^{-1})	Vibrational mode	Assignment
747	Ring and γ(C–O–H) of COOH	Pectin
969	$\delta(CH_2)$	Aliphatics
1000	C–CH_3 stretching	Carotenoids and proteins
1048	ν(C–O) + ν(C–C) + ν(C–O–H)	Cellulose and phenylpropanoids
1115	ν(C–O–C) and δ(C–O–H)	Cellulose
1155	C–C stretching, asymmetric ring breathing	Carotenoids and cellulose
1185	ν(C–O–H) Next to aromatic ring + δ(CH)	Carotenoids
1218	δ(C–C–H)	Carotenoids and xylan
1265	Guaiacyl ring breathing, C–O stretching	Phenylpropanoids and unsaturated fatty acids
1286	δ(C–C–H)	Aliphatics
1326	$\delta(CH_2)$ bending	Cellulose and phenylpropanoids
1339	ν(C–O) and δ(C–OH)	Carbohydrates
1440	$\delta(CH_2)$ + $\delta(CH_3)$	Aliphatics
1525	C=C stretching	Carotenoids
1606	ν(C–C) aromatic ring	Phenylpropanoids
1682	COOH	Carboxylic acids
1748	C=O stretching	Esters, aldehydes, carboxylic acids and ketones

1525 cm^{-1} were assigned to carotenoids, a group of pigments responsible for yellow, orange, and red coloration in plants. The band at 1155 cm^{-1} corresponds to C–C stretching and asymmetric ring breathing, while the band at 1526 cm^{-1} is attributed to C=C stretching vibrations in the conjugated carbon chain of carotenoids.

Aromatics and carbohydrates: The peak at 1608 cm^{-1} assigned to aromatic rings and the 1.265 cm^{-1} band attributed to guaiacyl ring breathing, suggesting phenylpropanoid compounds. Aromatic compounds were further supported by the 1,748 cm^{-1} peak, corresponding to C=O stretching, which points to esters and aldehydes, among other functional groups. Peaks related to carbohydrates were also detected like the 1,048 cm^{-1} band, which was assigned to C=O and C–O–H stretching vibrations, indicating carbohydrate content in the plant leaves.

Raman spectra of medicinal plants

F. deltoidea: Commonly known as mistletoe fig, is a small evergreen shrub or tree that belongs to the Moraceae family and native to Southeast Asia. Three key Raman peaks are highlighted for *Ficus deltoidea* leaf; 747 cm^{-1}, 1,155 cm^{-1} and 1,525 cm^{-1}, each corresponding to apectin, carotenoids/cellulose and carotenoids, respectively (Fig. 2).

L. inermis: Commonly known as henna, is a flowering plant belonging to the family Lythraceae and native to northern Africa, western and southern Asia. For *L. inermis,* other than carotenoid band at 1,526 cm^{-1}, there was the prominent peak at 1,748 cm^{-1} associated with the C=O stretching vibrations of carbonyl groups found in esters, aldehydes, carboxylic acids, and ketones. Further, phenylpropanoids or major phenolic band was evident at 1,608 cm^{-1} (Fig. 3).

M. citrifolia: Commonly known as noni, is a tropical plant belonging to the Rubiaceae family and native to Southeast Asia, the Pacific Islands and Australasia. Major bands contributed to noni leaf were carotenoids, cellulose and phenylpropanoids at 1,185 cm^{-1}, 1,218 cm^{-1} and 1,265 cm^{-1} bands (Fig. 4).

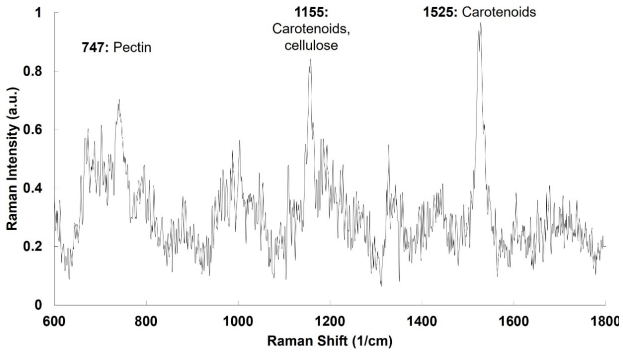

Fig. 2: Raman spectra of *F. deltoidea* var. *deltoidea* (Mistletoe fig) leaf

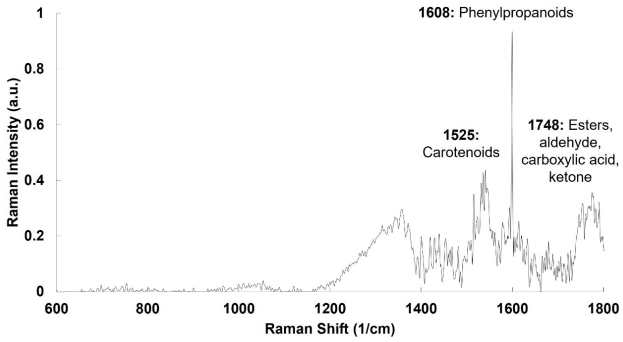

Fig. 3: Raman spectra of *L. inermis* (Henna) leaf

Discussion

The leaves used in this study were taken from *F. deltoidea* (mistletoe fig), *M. citrifolia* (noni) and *L. inermis* (henna)

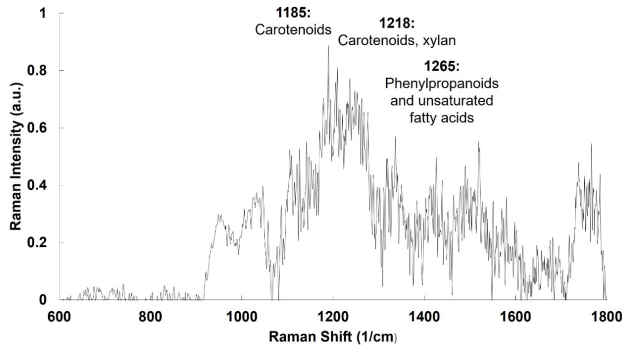

Fig. 4: Raman spectra of *M. citrifolia* var. *citrifolia* (Noni) leaf

plants due to their usage as medicinal plants particularly in tropical and sub-tropical areas. Different functional groups presented in phytochemicals such as aromatics (ring), carbonyl (C=O) and alkyl (C-H) groups produced specific Raman peaks. Phytochemicals like flavonoids and phenolic compounds that contain aromatic rings are key components to medicinal plants that provide health benefits to human (Sun and Shahrajabian 2023).

The leaves assessed in this study have bands of photosynthetic pigments including carotenoids, which consistent with previous study showing the detection of carotenoids in tea leaves using Raman spectroscopy (Payne *et al*. 2024). Each of the Raman spectra of the respective plant leaves are unique; however, they possess similarities, which all of the spectra had a minimum of one carotenoid and one aromatic as prominent band. Carotenoids are vital pigments found in the chloroplasts and chromoplasts of plants and protect plant cells from oxidative damage by quenching reactive oxygen species (ROS) produced during photosynthesis (Qi *et al*. 2025).

On the other hand, aromatic compounds such as phenolics and flavonoids are abundant in medicinal leaves. The primary roles of the compounds including defence against herbivores and pathogens**,** UV protection, and antioxidant activity highlight the plant's biological processes and their potential health benefits for humans (Sun and Shahrajabian 2023). *F. deltoidea* leaves have been widely used for its medicinal properties including wound healing and antidiabetic. The presence of aromatic peaks was due to the presence of phytochemicals including gallic acid, catechin, p-coumaric acid and vitexin (Hasnat *et al.* 2024). Further, it was expected for high content of cell wall materials including pectin and cellulose for *F. deltoidea* leaf as it is known to have thick leaves.

In the case of *L. inermis* plant, or henna, showed a very prominent and sharp peak at 1608 cm^{-1}, which revealed the presence of major compounds from phenylpropanoids pathways including polyphenols, naphthoquinone derivatives, lawsone and flavonoids (Batiha *et al*. 2024). Lastly, the plant *M. citrifolia*, or noni, has been used for centuries in traditional medicine, especially in Polynesian, Indian, and Southeast Asian cultures, where various parts of the plant (fruits, leaves, roots and bark) have been utilized for their health-promoting properties (Hou *et al*. 2025). *M. citrifolia* leaves are known to be rich in bioactive compounds predominantly of gallic acid, orientin, rutin and ferulic acid (Zhou and Huang 2024).

Recently, Raman spectroscopy has been used in a number of plant research. For instance, Raman scattering intensity of cutin, polysaccharides, phenolics and flavonoids was used to evaluate developmental stages of tomato fruit (Moreno *et al*. 2023). Son and team identified stress signalling molecules in real-time including salicylic acid, extracellular adenosine triphosphate, cruciferous phytoalexin and glutathione in watercress, common wheat and barley using surface-enhanced Raman scattering (SERS)-based nanoprobe (Son *et al*. 2023).

In addition, Raman spectroscopy allowed for detection of healthy or diseased kiwi leaves using carotenoid band at 1520 cm^{-1} (Jiang *et al*. 2023) and toxic hexavalent chromium (Cr^{6+}) in tea leaves using 595 cm^{-1} band assigned to the vibration of C–N–S band simultaneously after redox reaction with methimazole (Yin *et al*. 2023). Thus, Raman spectroscopy helps ensure the quality in terms of the presence of secondary metabolites, stress signalling molecules and toxic materials in plants by rapidly detecting and quantifying key phytochemicals.

This study highlights the use of Raman spectroscopy in the rapid and direct identification of associated phytochemicals presented in *F. deltoidea, L. inermis* and *M. citrifolia* plant leaves. There are limitations to this study that future research should address. In this study, the Raman spectra obtained only from particular spatial distribution of the leaves as some phytochemicals may accumulate in several locations of the leaves. Additionally, the study did not use to any standard bioactives presented in the leaves as the study's scope is to detect the presence of phytochemicals with rapid and direct detection utilizing Raman spectroscopy. Therefore, additional research on specific phytochemicals across a broader distribution on leaf parts is necessary to understand the role of Raman spectroscopy in medicinal plants.

Conclusion

This study reported the presence of prominent aromatics, carotenoids and phenylpropanoids Raman bands in medicinal plant leaves including *F. deltoidea*, *L. inermis* and *M. citrifolia* representing the therapeutic benefits of the leaves. Raman spectroscopy allows for rapid analysis without or less sample preparation to determine valuable phytochemicals and quality of plant leaves, thus making it an efficient tool for evaluating the quality and phytochemical abundance of medicinal plants. The extension of this work by using Raman spectroscopy would allow for the rapid detection of beneficial therapeutic substances and create more efficient systems for producing high-quality medicinal plants.

Acknowledgements

The author thanks University Utara Malaysia for financial support (21217).

Author Contributions

The author planned the experiments, analyzed the data, made illustrations and write up.

Conflict of Interest

The author declares no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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