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RESEARCH ARTICLE

Comparative analysis of FTIR profile in different species of *Thunbergia*

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Abstract

Thunbergia is a plant genus with many medicinal properties, such as anti-inflammatory, hepatoprotective, antipyretic, and detoxicant. It was traditionally known to be used as a medicine by rural and tribal people of Asia and Africa. The present study used Fourier Transform Infrared (FTIR) spectroscopy for characterisation and comparative study of bioactive compounds and functional groups' presence in the different parts of the *Thunbergia* plant. Three parts of the plant, i.e. leaf, stem and flower, were studied in three different species, i.e., *T. alata*, *T. erecta* and *T. mysorensis*.

FTIR spectroscopy checks the presence of various functional groups in the sample under study, such as carboxylic acid, alkane, aldehyde, alkynes, aliphatic amines, etc. Our findings exhibit that different parts of *Thunbergia* have different medicinally important bioactive constituents with different functional groups. It justifies its use as a traditional medicine and promises its application in modern medicine.

In the study, the major functional groups reported in the leaf extract were hydroxyl groups, lipids, alkanes, secondary amines, fatty acids, benzenoid compounds and phenols. This research shows that *Thunbergia* is a potential source of antioxidants and provides an opportunity for developing natural products from *Thunbergia* in drug discovery.

Keywords: *Thunbergia alata*, *Thunbergia erecta*, *Thunbergia mysorensis*, Phytochemicals, Fourier transform infrared spectroscopy, Traditional medicine

Introduction

Plants play a vital role in the life of all living organisms, including humans. The various roles of plants in the life of humans vary from providing food, shelter, environmental goods to other economically important materials. Additionally, one significant contribution of plants to human life is their medicinal significance, i.e., helping humans get rid of diseases at the level of prevention and cure. (Sofowora, 1993; Wilaiwan, 2024).

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More than 80% of the world population reportedly rely on traditional medicines for primary health care, and most therapies use plant extracts and their active compounds (Winston, 1999). India is the birthplace of the renewed indigenous medicine system, which includes Siddha, Ayurveda, and Unani. Traditional systems of medicines were based on plant parts, and their efficacy depends on the use of proper plant parts and their biological activities, which in turn depends on the presence of the required quantity and nature of secondary metabolite in a raw drug (Vinoth *et al*., 2011 and Savithramma *et al*., 2010). Ayurveda from India is one such concept. The studies suggest that two-thirds of all plant species have some medicinal value (Krishnaiah, 2011).

However, plants are living organisms comprising various organic molecules; only specific molecules are responsible for providing medicinal properties. So, to study how plants help humans provide medicinal inputs, researchers must filter out the molecules responsible for such properties. Without screening the active compounds from plants, a researcher can't invent a new medicinal drug to cure the target disease.

One of the plant groups that is reported to have high medicinal properties is *Thumbergia*. *Thunbergia* is a genus

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of flowering plants belonging to the family Acanthaceae. The plant family Acanthaceae constitutes more than 100 species (Burg, 2008) of robust annual or perennial vines which grow almost 2-8 m long. It is widely distributed as a shrub or climber. *Thunbergia erecta* is commonly known as King of the Mantle. This plant is primarily found in tropical and subtropical regions. i.e., India, Pakistan, Burma, Sri Lanka, Phillippines and most African countries.

The plants under this taxon are well-known for their ornamental and medicinal properties. These plants are reported to have various bioactive compounds, such as flavonoids, terpenoids, and phenolic acids, which are traditionally used in ethnomedicine. This plant has insecticidal, anti-inflammatory, hydragogue, sudorific, diuretic, and pesticidal properties. Fresh leaf juice is used for ringworm, snakebite, scorpion bite, skin diseases, impetigo, syphilis sores, itching, mycosis (washerman's itch), herpes and eczema. This plant's leaves and flowers possess many biological properties, such as antibacterial, antifungal, anti-inflammatory, antitumor, and expectorant. They are also helpful in treating urinary tract problems (Quattrocchi, 2012).

Though the plant and its parts are traditionally used for medicinal purposes, significant chemical studies on bioactive compounds were still lacking. Identifying the chemical composition of these compounds in plants is crucial for understanding their pharmacological potential. Fourier Transform Infrared (FTIR) spectroscopy is a widely used technique to analyse plant materials for the presence of chemical bonds and functional groups.

Of the various techniques, FT-IR spectroscopic analysis is considered an effective, powerful technique for investigating phytochemical groups and characterising chemical bonds present in biological samples (Annette, 2014; Anil, 2016; Charushila, 2016).

The *Thunbergia* plants have been selected to screen for possible bioactive compounds in the present research work. With this objective, FTIR analysis was done on the plant extracts from three parts (leaf, stem, and root) of three species of *Thunbergia*. The primary aim of the present study on *Thunbergia* was to investigate the main functional components of different phytochemical groups present in the different parts of this medicinally important plant.

By analyzing the differences and similarities in the spectra, this research seeks to provide insights into the molecular composition of these species and their potential pharmacological properties.

Materials and Methods

Preparation of plant sample

Three species of genus *Thunbergia,* i.e., *T. alata*, *T. erecta* and *T. mysorensis,* were selected to study their functional groups. The plant material was grown in the garden of the Botany Department, C. C. S. University, Meerut, and later harvested as and when needed. At the time of flowering, the plant parts, i.e. leaves, stem and flower, were separated. Before analysis, the samples were washed thoroughly in tap water to remove any dust particles and finally rinsed in distilled water. All plant samples were air-dried at room temperature on clean blotting sheets. The wholly dried samples were powdered using a regular grinder and stored in air-tight containers for further studies. When all the samples were ready, they were subjected to FTIR Spectroscopic analysis. All spectra were recorded on an Agilent FTIR Spectrometer (ATR module of carry 630 FTIR, Agilent Technologies).

The powdered samples of the plant parts in small amounts were directly analysed in FTIR spectrometer with constant application, and data of infrared absorbance were collected over the wave number that ranged from 600- 4000 cm^{-1} with a resolution of 2 cm^{-1}. The FTIR spectrum of all samples was analysed based on peak values in the infrared radiation region.

FTIR Spectroscopy

Many biophysical techniques can be used to study and characterize several phytochemical compounds in plant extracts. One of the standard methods is FTIR spectroscopy. FTIR Spectroscopy is feasibly the most potent tool for identifying the type of functional group (chemical bond) present in the extract (Pakkirisamy 2017; Sandip *et. al*., 2023). It is used to identify the functional groups in gaseous, liquid and solid materials via infrared radiation beams (khan *et al*., 2018). FTIR is used to identify biomolecules in different plant species like *Erythrina variegata* (Hemmalakshmi *et al*., 2017), *Myristica dactyloides* (Rajiv *et al*., 2017) and *Urtica dioica* (Maobe and Nyarango 2013).

FTIR spectra were recorded using a standard FTIR spectrometer of 4000–400 cm^{-1}. The wavelength of the light absorbed is characteristic of the chemical bond. The spectra were analysed to identify key functional groups based on the absorption bands. By interpreting the infrared spectrum, the functional groups in a molecule can be determined (Sahayaraj 2015).

Results and Discussion

FTIR spectrum was used to identify the plant extract's functional group of active components. Many researchers applied the FTIR spectrum as a tool for distinguishing closely associated plants and other organisms (Lu H-F *et al*., 2004; Kim SW *et al*., 2004; Lamprell *et al*., 2006; Rebuffo *et al*., 2006 and Sahoo *et al*., 2011). FTIR is a high-resolution analytical technique that helps identify critical chemical constituents in plants and elucidate the structural components (Aysal *et al*., 2007; Ibrahim *et al*., 2008).

The result of FTIR analysis confirms the presence of various chemical constituents such as hydroxyl group, carboxylic acid, alkane, aldehyde, nitriles, alkyne, isothiocyanate, alkene, nitro compound, aliphatic amines, halogen compound in different parts of *Thunbergia* species.

Table 1: FTIR frequency range, intensity, and functional groups of different plant parts of *Thunbergia alata*

Functional group	*Peak position (cm^{-1})*	*Possible assignment*	*Intensity (leaf)*	*Intensity (stem)*	*Intensity (flower)*
O-H Stretching (Hydroxyl)	3274-3337	O-H stretching (alcohols, phenols, H-bonding)	3274.4 - Strong	3337.7 – Strong	3288.5 – Strong
C-H Stretching (Alkane)	2918-2921, 2852-2856	C-H stretching (methyl, methylene)	2919.9 – Medium 2856.2 – Medium		
C=O Stretching (Carbonyl)	1604-1728	C=O stretching (aldehydes, ketones, esters)	1726.6 - Strong	1728.2 – Medium	1724.7 – Medium
Aromatic C=C Stretching	1604-1618	C=C stretching (aromatic rings)	1617.9 – Strong		
Fingerprint Region (C-O)	1233-1406	C-O stretching (alcohols, esters, phenols)	1232.7 - Medium		1368.4 – Medium
Unique Bands (C-H Bending)	780-1027	C-H out-of-plane bending (aromatics)	-	-	-

Table 2: FTIR frequency range, intensity, and functional groups of different plant parts of *Thunbergia erecta*

Functional group	*Peak position (cm^{-1})*	*Possible assignment*	*Intensity (leaf)*	*Intensity (stem)*	*Intensity (flower)*
O-H Stretching (Hydroxyl)	3274-3337	O-H stretching (alcohols, phenols, H-bonding)	3274.4 (leaf) - Strong	3322.8 (stem) - Strong	3284.9 (flower) – Strong
C-H Stretching (Alkane)	2918-2921, 2852-2856	C-H stretching (methyl, methylene)	2918.1 – Medium 2852.3 – Medium		
C=O Stretching (Carbonyl)	1604-1728	C=O stretching (aldehydes, ketones, esters)	1735.1 (leaf) - Strong	1729.4 (stem) - Medium	1727.3 (flower) – Medium
Aromatic C=C Stretching	1604-1618	C=C stretching (aromatic rings)	1604.0 (leaf) – Medium 1617.9 (leaf) – Strong		
Fingerprint Region (C-O)	1233-1406	C-O stretching (alcohols, esters, phenols)	1232.7 - Medium	1237.6 - Medium	1406.2 (flower) – Medium
Unique Bands (C-H Bending)	780-1027	C-H out-of-plane bending (aromatics)	-	-	

Table 3: FTIR frequency range, intensity, and functional groups of different plant parts of *Thunbergia mysorensis*

Functional group	*Peak position (cm^{-1})*	*Possible assignment*	*Intensity (leaf)*	*Intensity (stem)*	*Intensity (flower)*
O-H Stretching (Hydroxyl)	3274-3337	O-H stretching (alcohols, phenols, H-bonding)	3279.5 - Strong	3331.9 – Strong	3275.9 – Strong
C-H Stretching (Alkane)	2918-2921, 2852-2856	C-H stretching (methyl, methylene)	2919.2 – Medium 2854.1 – Medium		
C=O Stretching (Carbonyl)	1604-1728	C=O stretching (aldehydes, ketones, esters)		1727.4 – Medium	1728.7 – Medium
Aromatic C=C Stretching	1604-1618	C=C stretching (aromatic rings)	1604.5 (leaf) – Medium		
Fingerprint Region (C-O)	1233-1406	C-O stretching (alcohols, esters, phenols)	1233.5 (leaf) - Medium 1410.8 (leaf) – Medium		
Unique Bands (C-H Bending)	780-1027	C-H out-of-plane bending (aromatics)	780.7 (leaf) – Weak		

The results showed 32 peaks indicating the presence of bioactive compounds such as sulfates, sulfonamides, sulfones, sulfonyl chlorides, alkanes, aromatic, alkenes, ester, ketenes, isocyanates, isothiocyanates, acetylene, nitrile, phosphine, aldehyde, amide and alcohol.

Table 1-3 summarises the FTIR data of *Thunbergia alata*, *Thunbergia erecta*, and *Thunbergia mysorensis* according to

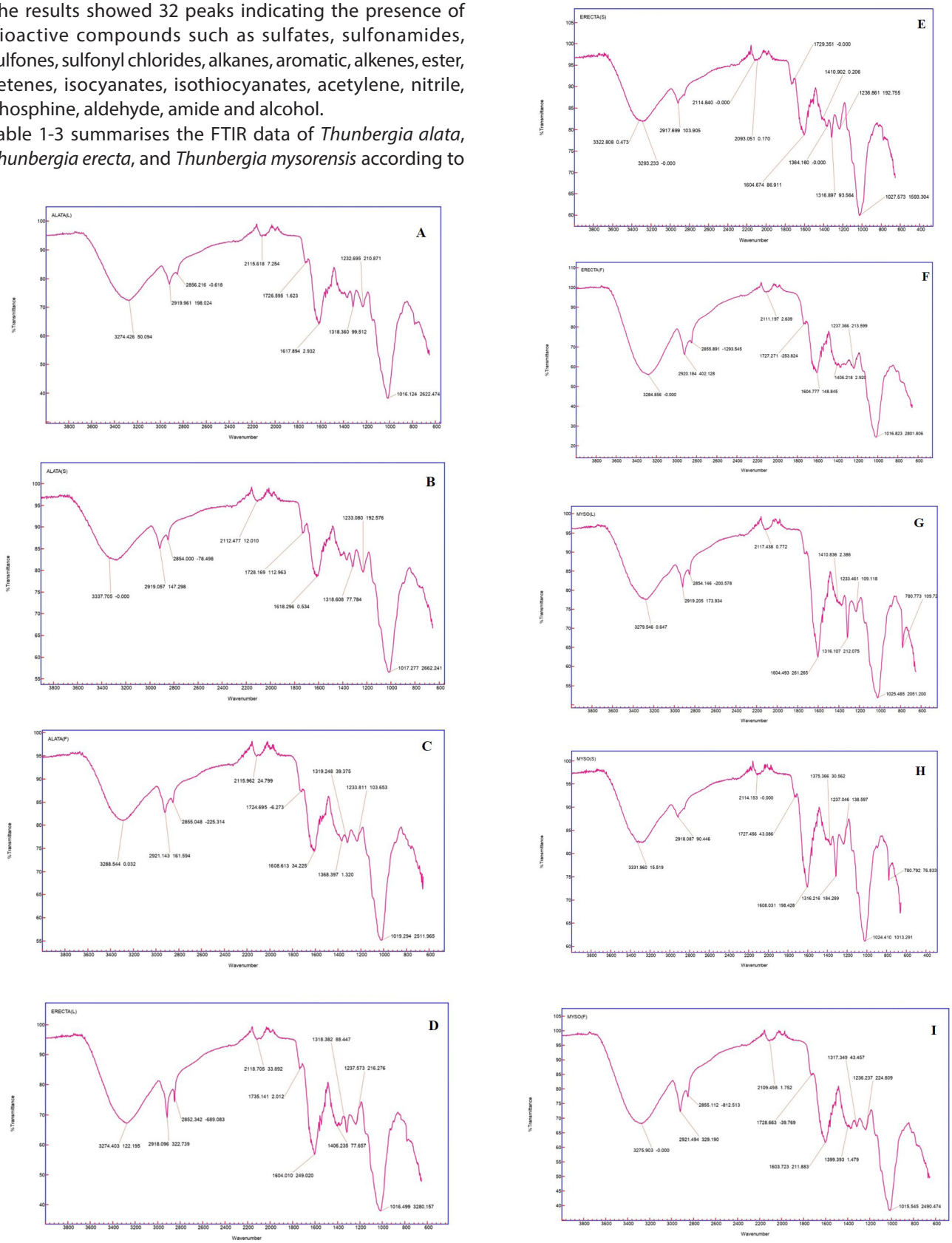

Figure 1 (A-I): show the observed spectra of different Thunbergia species, i.e., **A:** *T. alata leaf*, **B:** *T. alata stem*, **C:** *T. alata flower*, **D:** *T. erecta leaf*, **E:** *T. erecta stem*, **F:** *T. erecta flower*, **G:** *T. mysorensis leaf,* **H:** *T. mysorensis stem* and **I:** *T. mysorensis flower*

their functional groups and the corresponding IR absorption bands.

The FTIR spectra analysis of *Thunbergia alata*, *Thunbergia erecta*, and *Thunbergia mysorensis* provides insights into the functional groups in different parts of these species, i.e., leaf, stem, and flower. These spectra allow us to identify the presence of key chemical compounds that may play a role in the pharmacological properties of these plants. A detailed interpretation of the FTIR absorption bands and their associated functional groups is provided below.

A strong absorption band is observed in the 3274-3337 cm^{-1} region across all three species, corresponding to O-H stretching, a characteristic of hydroxyl groups commonly found in alcohols and phenolic compounds. These groups are often present in compounds like flavonoids and phenolic acids, which are well-known for their antioxidant properties. In *Thunbergia alata*, the leaf (Figure 1A) shows an absorption band at 3274.4 cm^{-1}, while the stem (Figure 1B) exhibits a slightly higher peak at 3337.7 cm^{-1}, and the flower (Figure 1C) exhibits peak at 3288.5 cm^{-1}. The broadness of these peaks indicates strong hydrogen bonding, possibly due to the presence of phenolic compounds, which are abundant in the leaves and flowers. Similarly, *Thunbergia erecta* shows O-H stretching peaks at 3274.4 cm^{-1} (Figure 1D), 3322.8 cm^{-1} (Figure 1E), and 3284.9 cm^{-1} (Figure 1F). These slightly lower values than *T. alata* may indicate variations in hydroxyl group density or differences in the hydrogen bonding environment. *Thunbergia mysorensis* presents similar peaks at 3279.5 cm^{-1} (Figure 1G), 3331.9 cm^{-1} (Figure 1H), and 3275.9 cm^{-1} (Figure 1I). The consistent O-H stretching peaks across all species suggest that hydroxyl-rich compounds, possibly flavonoids and tannins, are present in comparable amounts. However, their molecular interactions differ slightly, as the varying absorption frequencies indicate. This strong presence of O-H groups across all three species suggests that these plants likely possess antioxidant activities due to polyphenolic compounds, which can scavenge free radicals, contributing to their medicinal value.

The absorption bands observed in the 2918-2921 cm^{-1} and 2852-2856 cm^{-1} range correspond to C-H stretching in alkanes, primarily associated with the presence of lipophilic compounds such as fatty acids and terpenoids. These vibrations are characteristic of methyl (CH_3) and methylene (CH_2) groups. *Thunbergia alata* displays peaks at 2919.9 cm^{-1} and 2856.2 cm^{-1}, suggesting the presence of aliphatic hydrocarbons, possibly in the form of waxes or terpenoids. These compounds are essential for the protective and defensive functions of plants. *Thunbergia erecta* shows slightly lower values at 2918.1 cm^{-1} and 2852.3 cm^{-1}. The shift in peak position compared to *T. alata* may reflect differences in chain length or branching of the hydrocarbons, leading to altered stretching frequencies. *Thunbergia mysorensis* presents C-H stretching vibrations at 2919.2 cm^{-1} and 2854.1 cm^{-1}, indicating similar hydrocarbon content. These slight variations in the peaks suggest subtle differences in the lipid profiles among the species.

These C-H stretching vibrations highlight the existence of lipidic or fatty components in the plant tissues, which could contribute to their hydrophobic properties. Such compounds may play a role in the plant's defence against microbial infections and insect predation.

The peaks observed between 1604 and 1728 cm^{-1} are attributed to C=O stretching vibrations, typically found in carbonyl-containing compounds such as aldehydes, ketones, carboxylic acids, esters, and lactones. These functional groups are crucial components of many bioactive molecules, including flavonoids, terpenoids, and phenolic acids, all known for their anti-inflammatory and antioxidant activities. *Thunbergia alata* shows absorption peaks at 1726.6 cm^{-1} (leaf), 1728.2 cm^{-1} (stem), and 1724.7 cm^{-1} (flower), indicating the presence of carbonyl compounds in all parts of the plant. The slight variation in peak values between plant parts suggests differing concentrations or forms of carbonyl-containing compounds, such as carboxylic acids or esterified phenolics. *Thunbergia erecta* exhibits peaks at 1735.1 cm^{-1} (leaf), 1729.4 cm^{-1} (stem), and 1727.3 cm^{-1} (flower). The higher peak in the leaf compared to the other species may indicate a greater concentration of flavonoids or other carbonyl-containing secondary metabolites in this plant part. In *Thunbergia mysorensis*, the C=O stretching vibrations are detected at 1727.4 cm^{-1} (stem) and 1728.7 cm^{-1} (flower). The consistent C=O absorption across species points to the widespread presence of compounds such as esters or carboxylic acids, possibly contributing to the plant's medicinal effects.

The carbonyl group is a key feature of many bioactive compounds in plant defence and human health benefits. The high concentration of these compounds in leaves, stems, and flowers suggests that all plant parts could be used in traditional medicine for their therapeutic properties.

The peaks observed between 1604 and 1618 cm^{-1} are attributed to the C=C stretching of aromatic rings, characteristic of phenolic compounds such as flavonoids and tannins. These compounds are known for their anti-inflammatory, antioxidant, and antimicrobial properties. *Thunbergia alata* shows a distinct peak at 1617.9 cm^{-1} (leaf), indicating the presence of aromatic compounds. It suggests that the leaves may contain high levels of phenolics, which are known for their antioxidant potential. *Thunbergia erecta* exhibits a slightly lower peak at 1604.0 cm^{-1}, which may indicate a lower concentration of aromatic compounds compared to *T. alata*. *Thunbergia mysorensis* also peaks at 1604.5 cm^{-1}, similar to *T. erecta*. The presence of aromatic compounds in both species highlights their potential use as antioxidant and anti-inflammatory agents.

The presence of aromatic compounds across all three species suggests that these plants contain a range of phenolic compounds, which may explain their traditional use in treating various ailments, including inflammation and oxidative stress.

The region between 1233 and 1406 cm^{-1} is the fingerprint region, containing absorption bands indicative of complex molecular structures and functional groups, including C-O stretching in alcohols, esters, and phenols. These vibrations are crucial for identifying specific bioactive compounds present in the plants. In *Thunbergia alata*, a peak at 1232.7 cm^{-1} (leaf) suggests the presence of C-O bonds in phenolic acids or esterified flavonoids. *Thunbergia erecta* displays a peak at 1237.6 cm^{-1}, with a similar interpretation, indicating that phenolic compounds are also prevalent in this species. *Thunbergia mysorensis* shows a unique peak at 1410.8 cm^{-1} (leaf), which could indicate the presence of carboxylic acids or esters not found in the other two species.

The fingerprint region provides valuable insight into the molecular complexity of these species, particularly the presence of phenolic acids and esters that contribute to their pharmacological activities.

One unique feature of *Thunbergia mysorensis* is an absorption band at 780.7 cm^{-1}, which is absent in *T. alata* and *T. erecta*. This peak likely corresponds to out-of-plane bending vibrations of C-H bonds in aromatic structures, suggesting the presence of unique aromatic compounds in *T. mysorensis*. These compounds may contribute to the distinct medicinal properties of this species and warrant further investigation.

Conclusion

The FTIR spectra analysis of *Thunbergia alata*, *Thunbergia erecta*, and *Thunbergia mysorensis* reveals a range of functional groups, including hydroxyl, carbonyl, and aromatic C=C groups, that are common across all three species. However, variations in peak positions and intensities suggest differences in the concentration and composition of bioactive compounds in different plant parts. These differences likely contribute to the unique medicinal properties of each species—the presence of hydroxyl and aromatic groups in particular highlights. The unique peaks observed in *T. mysorensis* suggest the presence of different bioactive compounds that warrant further investigation**.**

Limitations and Prospects of the Research

The study was performed only on three species of *Thunbergia* genus. However, it was a preliminary study to confirm the presence of medicinally bioactive compounds. Some more species and some advanced chromatographic studies need to be done for further studies.

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