

COMPARATIVE FTIR SPECTROSCOPIC ANALYSIS OF *MORINGA OLEIFERA* LEAVES EXTRACTED IN POLAR AND NON-POLAR SOLVENTS: INSIGHTS INTO PHYTOCHEMICAL DISTRIBUTION AND SOLVENT SELECTIVITY

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Abstract

Moringa oleifera is a well-known traditional medicinal plant for its magical vitality. The medicinal effects are attributed to various bioactive compounds present in this plant species. The goal is to extract these bioactive compounds efficiently. In this study, various polarity gradient solvents were used to extract the compounds from the *M. oleifera* leaves. The extraction profile was analyzed by using comparative FTIR analysis in the mid-infrared region (4000–400 cm^{-1}). Spectral data revealed distinct variations in peak intensities and positions, corresponding to functional groups such as aldehyde, phenol, alkyl aryl ether, sulfoxide, alkane, conjugated acid, vinyl ether, alkene, amine salt, carboxylic acid, conjugated aldehyde, halo compound, aromatic compound, and alcohol. n-Hexane, chloroform, ethanol, and distilled water extracts exhibited more diverse and intense peaks, suggesting greater solubility of bioactive compounds. Leaves extracted with the remaining solvents contain fewer, but still important, functional groups. These findings provide insights into the solvent selectivity for tissue-specific phytochemical composition of *M. oleifera*, informing future applications in the pharmaceutical, nutraceutical, and cosmeceutical industries.

Keywords: *Moringa Oleifera*, FTIR Spectroscopy, Polarity Gradient Solvents, Phytochemical Profiling, Functional Groups, Industrial.

INTRODUCTION

Moringa oleifera is a fast-growing, miraculous tree commonly known as the drumstick tree. It is native to Africa and Asia. It is a worldwide-recognized species due to its benefits. *M. oleifera* has an enriched nutrient profile and exhibits therapeutic medicinal potentials. Therefore, it is considered a satisfactory candidate for industrial purposes (Pareek et al., 2023).

The whole *Moringa* plant parts are enriched with versatile bioactive phytochemicals, approximately over 100 characterized, including phenolics, flavonoids, glucosinolates, alkaloids, terpenoids, isothiocyanates, saponins, and vitamins, which make this plant potentially a stronger and effective carrier for different bioactivities such as anti-inflammatory, antidiabetic, antioxidant, antihelminthic, antimicrobial, cardioprotective, and anticancer (D. Kumar et al., 2022; Kashyap et al., 2022). These key features unravel *Moringa oleifera*'s importance in ethnopharmacology, functional food development, industry, and sustainable agriculture (Arshad, 2025). The phytochemical characterization is crucial to explore the biological effects of *Moringa oleifera* (Amin et al., 2024). There are numerous characterization techniques available, but among them, Fourier Transform Infrared (FTIR) spectroscopy is considered the most suitable candidate. FTIR is a rapid, cost-effective, and non-destructive characterization technique for determining functional groups and molecular signatures in complex biological matrices. It provides a thoughtful and reliable approach to gaining insight into key structural hierarchies of phytochemicals such as phenolics, saponins, flavonoids, thiocyanides, terpenoids, and alkaloids (Joshi et al., 2022; Govindan, 2018).

However, several studies have shown that phytochemicals from *M. oleifera* were extracted using single or limited solvent systems. So, there is a clear lack of using a complete gradient solvent system for the comprehensive comparative analysis employing FTIR spectroscopy (Sivapragasam et al., 2024; Obialo et al., 2024). This gap restricts our knowledge of natural chemical libraries, along with extraction strategies for targeted applications. It addresses the recent gap by straddling the entire polarity gradient, employing FTIR for each extract (from highly polar to intermediate and intermediate to less polar).

The combination of FTIR with optimized solvent extraction protocols along a polarity gradient scheme provides a deep insight into bioactive compounds fingerprinting in *M. oleifera* species, as well as enhancing the role of medicinal plants in nutraceuticals, cosmeceuticals, and pharmaceuticals (Kessler et al., 2023; Solomon et al., 2017; Baldisserotto et al., 2023).

MATERIALS AND METHODS

Sample Collection and Preparation

The *M.oleifera* plant leaves were collected from the city of Bahawalpur (29.103676, 71.736875) in Pakistan. The specimen was authenticated by Professor Dr. Mushtaq

Ahmad, Director of the Botanical Garden and Herbarium at Quaid-i-Azam University, Islamabad 45320, Pakistan. The specimens of *Moringa* leaves were stored with a voucher specimen number (No. 2112003). The dried *Moringa* leaves, shaded and carefully preserved in an airtight plastic bag, were kept in the fridge at 4°C for future use.

Solvent Extraction

By following the methodology of Mohammed et al. (2024), crude plant extracts were obtained with minor alterations. Initially, leaves of the *Moringa oleifera* plant were screened. The selected plant sample was washed and shadow-dried at room temperature to avoid the loss of any essential constituents and their activity for further investigations. After shadow drying, the collected sample was ground into a fine powder form for crude extract preparation. The extracts of the selected sample were prepared by increasing solvent polarity order; the solvents used for extraction included n-hexane, chloroform, ethyl acetate, acetone, ethanol, methanol, and distilled water. At room temperature, fine powders of the selected sample were separately macerated (1:10) in each of the solvents for about 72 to 168 hours. Afterward, the solutions were filtered using Whatman No. 41 filter paper. The plant filtrate was concentrated at room temperature and considered a crude extract. These extracts were stored at 4°C until FTIR analysis (Sinha et al., 2022).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

This analysis was carried out using a Fourier Transform Infrared Spectrophotometer (model: Spectrum Two, Perkin Elmer, M/S Analytical Measuring System, Singapore, SA (PC1)). One milligram of dried crude extract of the plant was deposited on the surface of the attenuated total reflection (ATR) crystal. Afterwards, the sample's surface was analyzed using Fourier Transform Infrared Spectroscopy (FTIR). To extract peaks, the solvent of the subject sample was set as the background to subtract its values from the acquired data. The subject sample was exposed to radiation. Spectrum range was calculated within the wavenumber 4000-400cm⁻¹. After taking spectrum measurements, the crystalline surface of FTIR was properly cleaned with demineralized water and gently dried with a paper towel (Sravan Kumar et al., 2015; Dev & Mukadam, 2025).

RESULTS

Comparative Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy of *Moringa oleifera* Lam. Leaves

ATR-FTIR is a rapid, non-destructive, widely used analytical technique for a very quick profiling of various functional groups and molecular signatures in plant extracts and drugs. FTIR analysis of plant extracts consistently reveals the presence of functional groups such as hydroxyl (O-H), carbonyl (C=O), carboxylic acids (C-O), aliphatic and aromatic (C-H), amines (N-H), and others. These functional groups correspond to major classes of bioactive compounds like phenolics, flavonoids, terpenoids, tannins, and alkaloids (Sahoo & Umashankara, 2023). The 600–1500 cm⁻¹ region of the FTIR spectrum is especially valuable for distinguishing plant species and identifying unique phytochemical

signatures (Wali et al., 2022). Absorption at different peak intensities reveals different functional groups of *Moringa oleifera* plant compounds. Various stretches suggest diverse bioactive compounds, N-H stretching for alkaloids, C-H stretching for terpenoids, while O-H stretching for flavonoids and polyphenols. The functional groups present in *Moringa oleifera* crude extracts are aldehydes, alkenes, amines, alcohols, phenols, aromatics, carboxylic acids, anhydrides, esters, ethers, and organic halogen compounds. Functional groups indicate the presence of various chemical groups in the *Moringa* plant species. The diversified chemical groups identified via FTIR analysis include phenols, alkyl aryl ethers, sulfoxide, alkane, conjugated acid, vinyl ether, alkene, amine salt, carboxylic acid, conjugated aldehyde, halo compounds, aromatic compounds, and alcohols. The *Moringa* functional groups diversification is attributed to various medicinal characteristics (functional groups mentioned in Table 1 and peak intensities in Fig. 1). The obtained FTIR results of *M. oleifera* provide a deep understanding of the phytochemical profile present in it, along with the comparative analog with other medicinal plants. Further purification and advanced analytical approaches are needed to fully characterize this magical medicinal herb for its miraculous benefits, such as chromatography, GC-MS, NMR spectroscopy, and mass spectrometry. The present study provides a deep insight into the spectral profile of *M. oleifera*, providing a crucial foundation for significant peaks and their intensities related to functional groups (Fig. 1).

Functional Groups Across Solvent Polarity (*Moringa* Plant Leaves-Specific Phytochemical Patterns)

The FTIR spectra of *M. oleifera* leaf are illustrated in Figures 1 (a-h). Corresponding data on the observed peak values and the associated functional groups identified through FTIR analysis are summarized in Table 1. These results provide insight into the chemical composition and diversity of functional groups present in the leaf part of *Moringa oleifera*.

FTIR of Unmacerated *Moringa oleifera* Leaves Powder

Table 1 and Figure 1a demonstrate the presence of 14 functional groups recognized from the dried, unmacerated powder of *Moringa oleifera* leaves. The strong peaks are identified at 1738 cm^{-1} , 1373 cm^{-1} , 1232 cm^{-1} , and 1044 cm^{-1} , which are assigned to the C=O stretching, O-H bending, C-O stretching, and S=O stretching vibrations. The other peaks also have remarkable functional group indications, which are assigned to the C-H stretching, C=O stretching, C-H bending, O-H bending, C-O stretching, C-F stretching, C=C bending, S=O stretching, C-Cl stretching, and C-Br stretching frequency vibrations. These groups were absorbed at different concentrations with different intensities. Their peak spectra indicate the presence of different secondary metabolites such as aldehyde, phenol, alkyl aryl ether, alcohol, and sulfoxide compounds.

The presence of these organic compounds poses a significant challenge in exploring their novelties in the realm of research. These compounds can be a remarkable breakthrough in beautifying and therapeutic applications with deep effects and less toxicity as compared to synthetic ones.

FTIR of n-Hexane Extract of *Moringa oleifera* Leaves

FTIR analysis revealed well-defined absorption bands corresponding to various functional groups at 2916 cm^{-1} , 2848 cm^{-1} (for C-H stretching) for the C-H group, at 1710 cm^{-1} and 1735 cm^{-1} for carbonyl groups (C=O), 1202 cm^{-1} and 1176 cm^{-1} (C-O stretching), and 983 cm^{-1} (C=C bending) exhibited by the n-hexane extract (see Table 1 and Fig 1b).

FTIR of Chloroform Extract of *Moringa oleifera* Leaves

The chloroform extract's characteristic absorption bands were observed at 2925 cm^{-1} , 2855 cm^{-1} (for C-H stretching), and 1710 cm^{-1} (for a carbonyl group, C=O), 1463 cm^{-1} (for C-H bending), 1215 cm^{-1} (for C-O stretching), 1167 cm^{-1} (for C-O stretching), 754.8 cm^{-1} (for C-Cl stretching), and 668.5 cm^{-1} (for C-Br stretching, see Table 1 and Fig 1c).

FTIR of Ethyl Acetate Extract of *Moringa oleifera* Leaves

The ethyl acetate extract's characteristic absorption bands were identified at 3365 cm^{-1} (O-H stretching), 2918 cm^{-1} , 2850 cm^{-1} (C-H stretching), 1713 cm^{-1} (C=O stretching), 1453 cm^{-1} (C-H bending), 1376 cm^{-1} (O-H bending), 1239 cm^{-1} (C-O stretching), and 1037 cm^{-1} (S=O stretching, see Table 1 and Fig 1d).

FTIR of Acetone Extract of *Moringa oleifera* Leaves

The acetone extract's characteristic absorption bands were identified at 1710 cm^{-1} (C=O stretching), 1421 cm^{-1} (O-H bending), 1359 cm^{-1} (O-H bending), 1221 cm^{-1} (C-O stretching), 1092 cm^{-1} (C-O stretching), 902.5 cm^{-1} (C=C bending), and 529.3 cm^{-1} (C-I stretching, see Table 1 and Fig 1e).

FTIR of Ethanol Extract of *Moringa oleifera* Leaves

The ethanol extract's characteristic absorption bands were identified at 3339 cm^{-1} (O-H stretching), 2974 cm^{-1} (C-H stretching), 1649 cm^{-1} (N-H bending), 1381 cm^{-1} (C-H bending), 1088 cm^{-1} (C-N stretching), 1045 cm^{-1} (C-N stretching), 879.1 cm^{-1} (C-H bending), and 602.1 cm^{-1} (C-Br stretching, see Table 1 and Fig 1f).

FTIR of Methanol Extract of *Moringa oleifera* Leaves

The methanol extract's characteristic absorption bands were observed at 3314 cm^{-1} (O-H stretching), 2943 cm^{-1} (C-H stretching), 2832 cm^{-1} (N-H stretching), 1449 cm^{-1} (C-H bending), 1022 cm^{-1} (C-N stretching), and 620 cm^{-1} (C-Cl stretching, see Table 1 and Fig. 1g).

FTIR of Distilled Water Extract of *Moringa oleifera* Leaves

The distilled water extract's characteristic absorption bands were observed at 3245 cm^{-1} (O-H stretching), 2886 cm^{-1} (C-H stretching), 1586 cm^{-1} (C=C stretching), 1412 cm^{-1} (S=O stretching), 1086 cm^{-1} (C-N stretching), 612.7 cm^{-1} (C-Br stretching), and 480.8 cm^{-1} (C-I stretching, see Table 1 and Fig 1h).

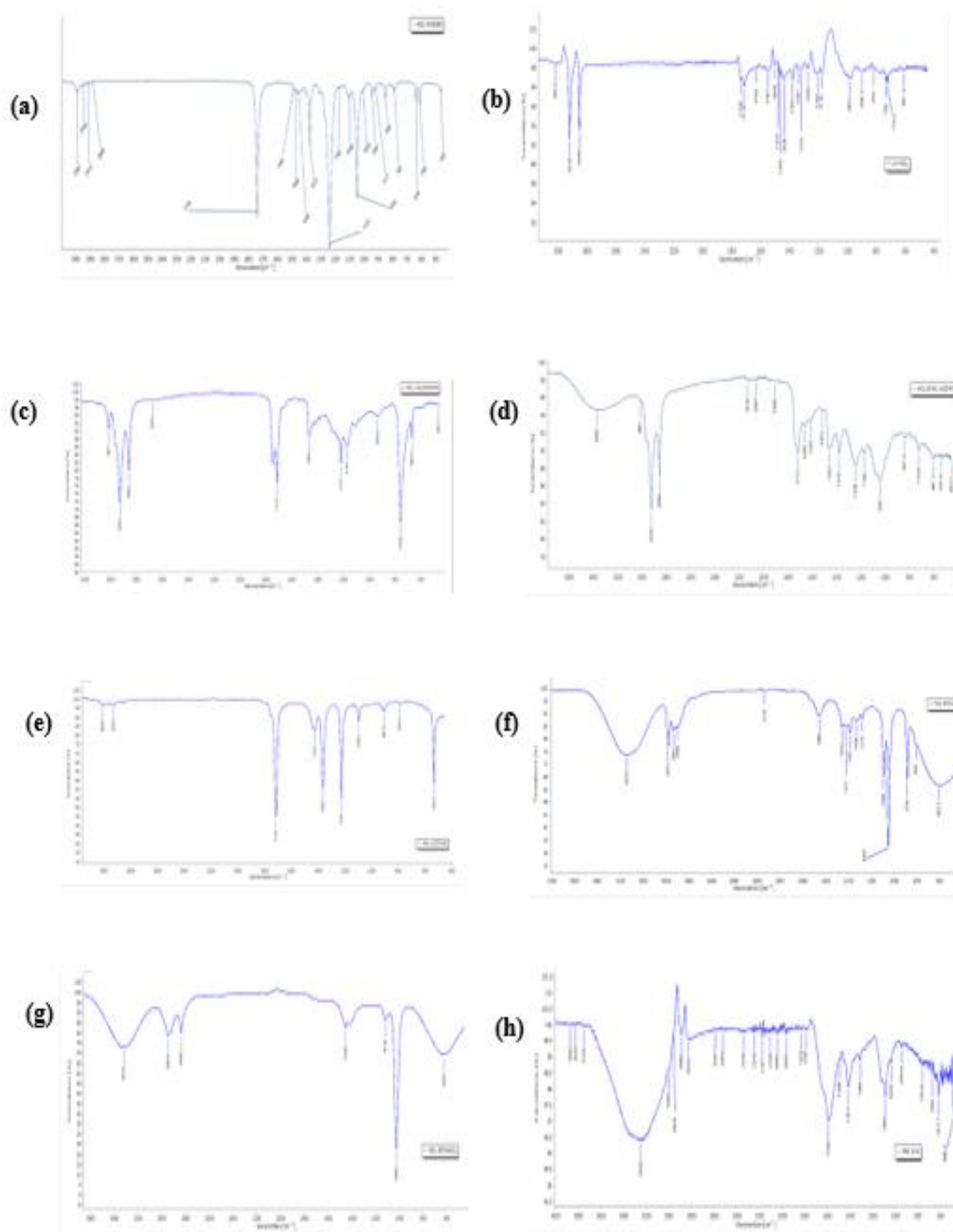


Figure1: FTIR Spectra of Moringa oleifera Leaves (a) Unmacerated Powdered Extract (b) n-Hexane Extract (c) Chloroform Extract (d) Ethyl Acetate Extract (e) Acetone Extract (f) Ethanol Extract (g) Methanol Extract (h) Distilled Water Extract

Table 1: FTIR Interpretation of Identified Compounds of *Moringa oleifera* Leaves

S.no	Wave number cm ⁻¹ (Test sample)	Wave number cm ⁻¹ (Reference Fig. 1)	Functional groups	Class	Peak Details
(a) Unmacerated <i>Moringa oleifera</i> Leaves Powder					
1	1738	1740-1720	C=O stretching	aldehyde	strong
2	1373	1390-1310	O-H bending	phenol	strong
3	1232	1275-1200	C-O stretching	alkyl aryl ether	Strong
4	1044	1070-1030	S=O stretching	sulfoxide	strong
(b) n-Hexane Extract of Leaves of <i>Moringa oleifera</i>					
1	2916	3000-2840	C-H stretching	Alkane	strong
2	2848	2830-2695	C-H stretching	aldehyde	strong
3	1735	1740-1720	C=O stretching	aldehyde	medium
4	1710	1710-1680	C=O stretching	conjugated acid	medium
5	1202	1225-1200	C-O stretching	vinyl ether	medium
6	983	980-960	C=C bending	alkene	medium
(c) Chloroform Extract of Leaves of <i>Moringa oleifera</i>					
1	2925	3000-2840	C-H stretching	alkane	strong
2	2855	3000-2800	N-H stretching	amine salt	strong, broad
3	2673	3300-2500	O-H stretching	carboxylic acid	medium
4	1710	1710-1685	C=O stretching	conjugated aldehyde	strong
5	754.8	850-550	C-Cl stretching	halo compound	strong
6	2925	3000-2840	C-H stretching	alkane	strong
(d) Ethyl Acetate Extract of Leaves of <i>Moringa oleifera</i>					
1	2918	3000-2840	C-H stretching	alkane	strong, broad
2	2850	3000-2800	N-H stretching	amine salt	strong, broad
3	1713	2000-1650	C-H bending	aromatic compound	strong, broad
4	1037	1070-1030	S=O stretching	sulfoxide	Strong and broad
(e) Acetone Extract of Leaves of <i>Moringa oleifera</i>					
1	1710	2000-1650	C-H bending	aromatic compound	strong
2	1359	1390-1310	O-H bending	phenol	strong
3	1221	1275-1200	C-O stretching	alkyl aryl ether	strong
4	529.3	600-500	C-I stretching	halo compound	strong
(f) Ethanol Extract of Leaves of <i>Moringa oleifera</i>					
1	3339	3550-3200	O-H stretching	alcohol	strong, broad
2	2974	3000-2840	C-H stretching	alkane	strong,
3	1045	1020-950	C-N stretching	amine	strong
4	1088	1250-1020	C-N stretching	amine	strong

5	879.1	900-860	C-H bending	1,2,4-trisubstituted	strong
6	602.1	690-515	C-Br stretching	halo compound	Strong and broad
(g) Methanol Extract of Leaves of <i>Moringa oleifera</i>					
1	3314	3650-2500	O-H stretching	carboxylic acid	strong, broad
2	1022	1250-1020	C-N stretching	amine	strong
3	620	850-550	C-Cl stretching	halo compound	Strong and broad
(h) Distilled Water Extract of Leaves of <i>Moringa oleifera</i>					
1	3245	3550-2700	O-H stretching	alcohol	strong, broad
2	1586	1650-1566	C=C stretching	cyclic alkene	Strong, broad
3	1412	1415-1380	S=O stretching	sulfate	Strong, broad
4	1086	1250-1020	C-N stretching	amine	Strong, broad
5	480.8	500-415	C-I stretching	Halo compounds	strong

DISCUSSION

The secondary metabolites are efficient in their therapeutic and pharmacological actions. Initially, active compounds are identified via preliminary profiling, leading towards more advanced diversified approaches. The pharmacologically categorized rich profile active compounds include alkaloids, tannins, flavonoids, saponins, and various other aromatic compounds and their derivatives. These active compounds not only belong to their pharmacological actions but also to the defense mechanisms against herbivory, insect predation, and microbial infections (Tousif et al., 2024). Hence, preliminary phytochemical characterization is a key step to unravel the complex diversity of bioactive compounds present in *Moringa* plant species, which provide foundational hierarchies for plants' biological actions (Aim et al., 2024).

According to a comprehensive study of Singh et al. (2022), a broad phytochemical profile of *Moringa oleifera* was identified with distinct functional groups attributed to major diverse chemical groups, which provides referenced foundations for medicinal hierarchies. Further, Hauck and Maheshwari point out the attention via FTIR spectroscopy towards *Moringa* functional groups diversification within the spectral range of 4000 to 400 cm^{-1} (Hauck et al., 2008; Maheshwari et al., 2023). Although substantial overlap was observed in the absorption spectra attributable to the complexity of the plant matrix, distinct bands corresponding to characteristic functional groups were still identifiable. These overlapping peaks reflect the collective contributions of multiple phytoconstituents (Joshi et al., 2022). Conclusively, the FTIR analysis provided valuable phytochemical markers that not only aid in quality assurance of the plant samples but also serve as a robust analytical tool for

the identification and standardization of medicinally important plant materials (Siahaan & Hamzah, 2023; Rajiv et al., 2017)

Gong explained in his findings that the Infrared region is important for identifying the key functional groups of active components in plant species. The absorption patterns of peaks are directly linked to the positions of compounds' functional groups falling in a specific spectral region (Gong et al., 2024).

The present findings explored diversified functional groups of chemical compounds in *Moringa oleifera* species through FTIR analysis, including alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatic compounds, esters, ethers, alkyl halides, and aliphatic amines, indicating the major phytochemical constituents (as detailed in Table 1). Additionally, the obtained results confirmed the presence of normal polymeric (OH stretch), alkyne (C-H stretch), methylene (C-H stretch), open-chain imino ($-C=N-$), organic nitrates, aliphatic nitro compounds, methyl (C-H) asym/sym bend, ammonium ions, aromatic nitro compounds, tertiary amine (C-N stretch), sulfides (S stretch), and halogen compounds. These findings are consistent with Adeyeni (Adeyeni et al., 2024). However, the obtained key findings suggest a deep understanding of the chemical nature of active metabolites of *Moringa oleifera* extracts, which can be used to build a stronger profile of pharmacological effects related to the relevant class of compounds.

Conclusively, the spectroscopic technique is relatively simple, cost-effective, and serves as a valuable tool for the rapid identification of functional groups (Jurina et al., 2023). In the present study, FTIR analysis of *M. oleifera* leaf extracts served as a pharmacognostic marker, enabling the distinction and authentication of this medicinally important species.

Our results are consistent with the findings of Sathish et al. (2016), who reported that the FTIR spectra of *Moringa oleifera* leaf extracts can be effectively used to identify and detect characteristic peaks corresponding to various functional groups of bioactive components within the infrared radiation region. Their study demonstrated the presence of phytochemicals produced during the plant's normal metabolic activities. Similarly, in the present study, *Moringa oleifera* leaf extracts were subjected to FTIR analysis, and the functional groups were identified based on their respective peak intensities and positions, confirming the presence of various bioactive compounds.

CONCLUSION

This study presents a comprehensive FTIR-based phytochemical profiling of *Moringa oleifera* leaves, extracted using a complete gradient solvent system from non-polar to highly polar. The findings establish clear solvent selectivity, with nonpolar solvents, predominantly n-hexane and chloroform, showing greater extraction proficiency for bioactive compounds as well with ethanol compared to others; such as aldehyde, phenol, alkyl aryl ether, sulfoxide, alkane, conjugated acid, vinyl ether, alkene, amine salt, carboxylic acid, conjugated aldehyde, halo compound, aromatic compound, alcohol.

Leaves were well-known, recognized, phytochemically rich organs, unveiling the paramount diversity and intensity of functional groups across the solvent gradient.

These patterns advocate substantial potential bioactivity, particularly antioxidant and antimicrobial properties, associated with the identified functional groups.

The results underscore the importance of solvent choice and plant part selection in phytochemical investigations, providing a foundational spectral reference for *M. oleifera*. Future work should focus on targeted isolation of individual compounds, bioactivity-guided fractionation, and integration with advanced analytical techniques such as LC-MS and GC-MS to further elucidate the therapeutic potential of specific phytoconstituents. Such approaches will augment the development of evidence-based solicitations in pharmaceuticals, functional foods, and natural health products.

Conflict of Interest

The authors report no conflict of interest regarding the present work.

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