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Evaluation of phyto-constituents of *Murraya koenigii* based on FTIR spectral data

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Abstract
Murraya koenigii (curry leaves) are a great source of valuable nutrients and bioactive compounds. Using Fourier Transform Infrared Spectroscopy, a quick and non-destructive analytical method, the current study sought to assess the phyto-constituents of curry leaves. Ethanol leaf extract was produced using a Soxhlet extraction method. Thin-layer chromatography was performed on the ethanolic leaf extract of *Murraya koenigii* to evaluate the solvent systems for separating the secondary metabolites. The mobile phases tested were n-propanol: formic acid: water (20:3:2), ethyl acetate: n-hexane (15:10), and glacial acetic acid: water: n-butanol (5:5:15), which were further applied for column chromatography. The Soxhlet-derived extract is subjected to column fractionation, and the collected fractions are used to identify functional groups in plant extracts by using Fourier Transform Infrared spectroscopy. From this study, it can be concluded that the extract consisted of various functional groups, including ether, alcohol, amine, alkyl halide, and aromatic ring, with characteristic absorption bands observed at 1078 cm^{-1}, 3458 cm^{-1}, 1592 cm^{-1}, 660-564 cm^{-1}, and 1567 cm^{-1} wavelengths, respectively, in the FTIR spectrum. The study will be explored for integration with other analytical techniques and quality applications, with prospects in advanced research, clinical validation, and herbal formulation.

Keywords: *Murraya koenigii,* Fourier transform infrared spectroscopy, thin-layer chromatography

Introduction
Murraya koenigii, commonly known as curry leaf, is renowned for its rich biodiversity of bioactive compounds and its longstanding use in traditional medicine across India (Salvi and Choudhary, 2020) [9]. It is known as "krishnanimba" in Indian Ayurvedic medicine (Aniqa and Kaur, 2024) [10]. The leaves, roots, bark, and fruit of the curry leaf tree are used for medicinal purposes (Aniqa and Kaur, 2024) [10]

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliospida
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae
Genus	Murraya J.Koenig ex L.
Species	*Murraya Koenigii* L. Spreng

Fig 1: Taxonomical status of Murraya koenigii

Murraya koenigii, also commonly known as Meethi neem (Kumari *et al.*, 2018) [6], is an aromatic, more or less deciduous shrub or a small tree up to 6 m in height found throughout

India up to an altitude of 1500 m and cultivated for its aromatic leaves (Iyer and Umadevi, 2008) [11]. The plant is utilized in various forms such as extracts, essential oils, or directly in its natural state owing to the presence of numerous bioactive constituents. These contain bioactive compounds like alkaloids (murrayamine, caryophyllene), phenolic acids (chlorogenic acid, gallic acid), tannins (proanthocyanidins, ellagitannins), and bismahanine, murrayanine, murrayafoline-A, bi-koeniquinone-A, bismurrayaquinone, mukoenines (A-C), murrastifoline, murrayazolinol, murrayacine, murrayazolidine, murrayazoline, mahanimbine, girinimbine, koenioline, xanthyletin, and koenigine-quinones A and B (Chaudhary, 2020) [12]. These constituents are secondary metabolites, which play a crucial role in the plant's pharmacological properties. These compounds influence various physiological processes and contribute to disease prevention and health maintenance (Balakrishnan *et al.*, 2020) [13]. *Murraya koenigii* leaf powder is rich in nutrients and exhibits favorable techno-functional properties, highlighting its potential application in food and nutraceutical formulations (Awari *et al*., 2023) [4].

Curry leaf (*Murraya koenigii*) is valued in Asian cooking for its flavor (Jain *et al.*, 2017) [1]. Plants produce two kinds of compounds: primary metabolites, which are needed for growth, and secondary metabolites, which help in defense and have important medicinal properties (Krishnaiah *et al*., 2009) [8]. Curry leaf has been traditionally used as a medicinal herb contains many phytochemicals that confer it antimicrobial, antioxidant, and anti-inflammatory properties (Rana and Yamini, 2022) [5]. *Murraya koenigii* bark contains bioactive phytochemicals, and these compounds were successfully separated and qualitatively analyzed using TLC (Anjaneyulu *et al*., 2017) [29]. Choosing the right solvents, stationary phases, and detection methods is important for getting the best separation in TLC (Thiyagarajan and Kanchana, 2023) [21]. FTIR is an important analytical method that provides a clear guide for interpreting spectra and can be used to analyze many forms of organic materials such as liquids, powders, films, and gases (Nandiyanto *et al.*, 2019) [2]. FTIR analysis aids in the identification of functional groups present in bioactive compounds by detecting the characteristic vibrational frequencies of molecular bonds, thereby providing critical insights into their structural composition and chemical nature [2, 3, 6, 18, 20]. The ethanol extract was not separated into individual chemical components before analysis, which restricted the results to an assessment of the entire mixture rather than specific compounds. There is no column chromatographic technique employed. This study aims to investigate the preparation of *Murraya koenigii* leaf extract. It will include conducting phytochemical tests and separating the compounds using thin-layer chromatography (TLC) with a specific cost-effective solvent system. Column chromatography will be utilized to obtain various fractions, which will subsequently be tested for antimicrobial activity. Finally, Fourier-transform infrared spectroscopy (FTIR) analysis will be performed on these fractions to identify their functional groups.

Materials and Methods

Plant collection and authentication

The leaves of *M. koenigii* were obtained from the local market, Kakinada, and were authenticated by a botanist at P.R. Govt. (A) College, Kakinada.

Chemicals

The study utilized several chemicals, including ethyl alcohol, ethyl acetate, formic acid, and silica gel-G, all of LR (laboratory reagent) grade. n-hexane, n-butanol, n-propanol, glacial acetic acid, and potassium bromide were employed in AR (analytical reagent) grade. All chemicals were sourced from the Department of School of Pharmaceutical Sciences and Technologies, Jawaharlal Nehru Technological University (JNTU), Kakinada, Andhra Pradesh.

Instruments

Bruker's FTIR-Alpha II, Essae-Teraoka's analytical balance, KEMI's BOD incubator (KBOD-65), Tempo's hot air oven (LS-121), hydraulic press (M-15), Borosil's reflex condenser, SOLTEC's ultrasonicator, Borosil's Soxhlet extractor, Guna Enterprises' heating mantle, Borosilicate Chromatography Column by Borosilicate Glass Works Ltd., round-bottom flask Borosil-250ml, and laminar air flow KLF-3SS (KEMI) were employed in the study.

Preparation of herbal powder

The curry leaves were dried in a hot air oven at a controlled temperature of 60 °C for 3 h. The dried leaves were then finely ground using a grinder and stored in a zip-lock bag at ambient temperature.

Preparation of extract Soxhlet extraction/Continuous hot percolation: [7, 9, 14, 18, 24]

Weighed 250 g of powdered *Murraya koenigii* leaves and placed them on filter paper. The filter paper was then positioned in a siphon tube. The Soxhlet apparatus condenser was filled with water, and extraction was performed using 250 ml of ethanol in a round-bottom flask (RBF). The extract was heated at 60 °C. The extract was concentrated via evaporation, allowing solvent vapor to condense and flow into the thimble with curry leaf powder. The system operated for approximately 7 h. The obtained crude ethanol extract was stored in a closed container and used for preliminary qualitative phytochemical analysis.

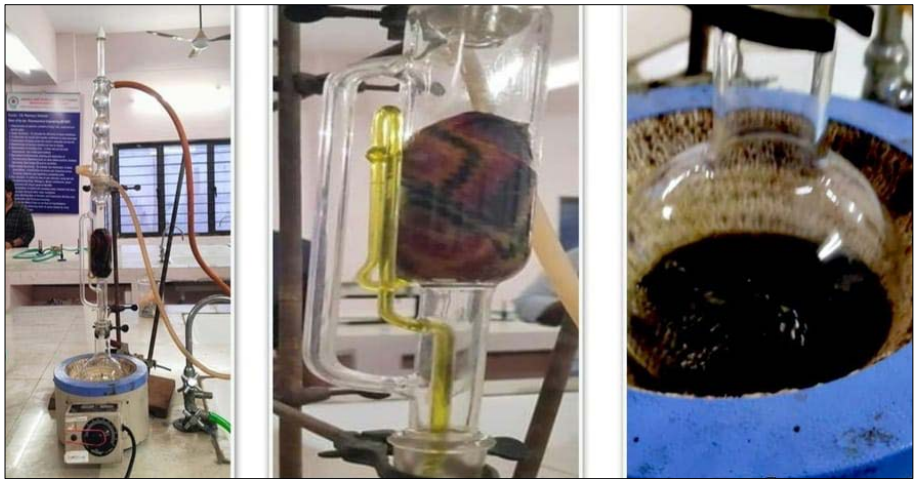

Fig 2: Soxhlet extraction of *Murraya koenigii* leaves was set up using ethanol as the solvent during the experiment.

Phytochemical Testing

Using the freshly prepared ethanol extract, phytochemical screening was carried out to examine the presence of tannins, quinones, phenols, and alkaloids. Table 1 outlines the standard qualitative procedures for each phytochemical test [5, 7, 15, 30].

Thin-layer chromatography

Using a pre-coated TLC plate, a small spot of the solution containing the sample, i.e., *Murraya koenigii* leaf extract, was applied on the pre-coated plate 1.0 cm from the bottom mark [7] using a capillary tube, ensuring the spots were small and evenly spaced. An appropriate solvent ratio for secondary metabolites was prepared to serve as the mobile phase. The TLC plate was positioned inside the developing chamber with the mobile phase, making sure that the solvent level remained below the applied sample spots. The solvent was allowed to pass through the plate by capillary action until it reached a predetermined height, known as the solvent front. Once this point was reached, the plate was removed from the chamber, and the spots of separated compounds were identified based on their corresponding frontal ratio (R_f) value [21]. Table 2 indicates the solvent systems used in the TLC method.

Antimicrobial activity

Test pathogens

The pathogenic bacteria used in this study was *E. coli* (ATCC 25922) [36]. The pure pathogenic strain was collected from the Department of School of Pharmaceutical Sciences and Technologies at Jawaharlal Nehru Technological University (JNTU), Kakinada, Andhra Pradesh. The collected pure culture was maintained on soyabean casein digest agar slants at 32-35 °C and subcultured for 24 h before use [33].

Cup plate method

Antibiotic Assay Medium no. 5 (Ciprofloxacin) was prepared and autoclaved at 121 °C for 15 min at 15 psi. A base layer (17 ml) was prepared and poured into sterile petri plates. After solidification of the base layer, the seed layer (medium inoculated with *E. coli*-4 ml) was poured over it and left at room temperature to set the media. Once the medium solidified, wells measuring 8 mm in diameter were created using a sterile cork borer. And well, it is loaded with 100 µl of ciprofloxacin antibiotic and 80 µl of *M. koenigii*'s extract; with the help of a micropipette, it diffuses in the medium and inhibits the growth of the organism. The plates were left for 1 h for pre-diffusion and incubated in a bacteriological incubator at 32-35 °C for 24 h. At the end of incubation, the zone of inhibition is measured around each well using a vernier caliper, and the sensitivity is determined [33].

Column chromatography: By using the solvent system obtained from TLC, column chromatography is performed as the dry method.

In this method, silica gel G functioned as the stationary phase [31], and the mobile phase was formulated using the solvent mixture applied in TLC. The solvent system for alkaloids consisted of n-propanol, formic acid, and water; for tannins, it consisted of glacial acetic acid, water, and n-butanol; and for phenols, it consisted of a blend of ethyl acetate and n-hexane. The ethanol extract was added after the dry silica gel had been filled into the column. The corresponding mobile phases were then added and allowed to saturate completely. To ensure appropriate separation, the process was kept in a wet condition. As the mixture passed through the column, its constituents exhibited varying interactions with the stationary phase, resulting in differences in migration rates and consequently leading to their separation. Each compound exited the column and was collected individually in fractions. Automated fraction collectors were used to organize the collection of these separated components [31, 32].

FTIR analysis for curry leaf extract

Pressed Pellet Technique: To prepare a sample for infrared (IR) spectroscopy, 1 gram of potassium bromide (KBr) and the separated fractions were placed separately in petri dishes. To eliminate residual moisture, the samples were subjected to heating in a hot air oven at 105 °C for 3 h. Once dried, the KBr and sample fragments were finely ground and mixed using a mortar and pestle to make a uniform powder. This mixture was compressed into a thin, transparent pellet by applying a pressure of 75 kg/cm^2 using a hydraulic press. A potassium bromide blank pellet was prepared to serve as a baseline reference.

The pellets of secondary metabolites were positioned on the FTIR spectrometer's sample holder and scanned 45 times across a spectral range of 4000-400 cm^{-1} with 2 cm^{-1} resolution. When IR radiation passed through the pellet, it

interacted with the molecules of the sample. Absorption occurred if the radiation energy corresponded to the vibrational frequencies of the molecules, producing an absorption spectrum that represented the extent of light absorbed at different wavelengths. By interpreting this pattern, the functional groups present in the sample were identified and provided with a molecular structure [3].

Results

Table 3 indicates the Phytochemical screening of *Murraya koenigii* extract

Thin-layer chromatography

Fig 3: Visual examination of Thin-layer chromatography of *Murayya koenigii* extract

R_f values: Table 4 indicates the R_f values for the separated phyto-constituents of *Murraya koenigii* extract.

Antimicrobial activity

Cup plate method: A visible zone of inhibition (ZOI) around each cup on the agar plate was observed. The extent of the inhibition zone was directly proportional to the antimicrobial potential, with larger zones reflecting greater activity. In this study, the drug (ciprofloxacin) and the curry leaf extract demonstrated higher antimicrobial activity**.** Table 5 demonstrates the zone of inhibition of curry leaf extract, ethanol, and ciprofloxacin.

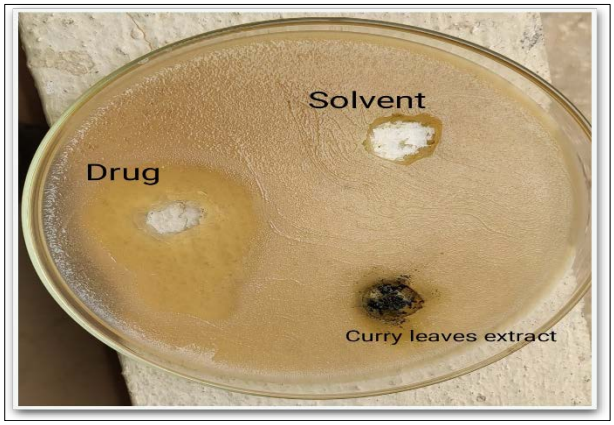

Fig 4: Antimicrobial activity of the ethanolic extract of curry leaves, ciprofloxacin, and ethanol was evaluated against *Escherichia coli*.

Pressed pellet technique: Pellets were prepared from the fragments obtained from the eluate of column chromatography and were heated in a hot air oven at 110 °C prior to analysis.

Fig 5: Pressed pellets of KBr and secondary metabolites of *M. koenigii* extract.

FTIR Spectrum

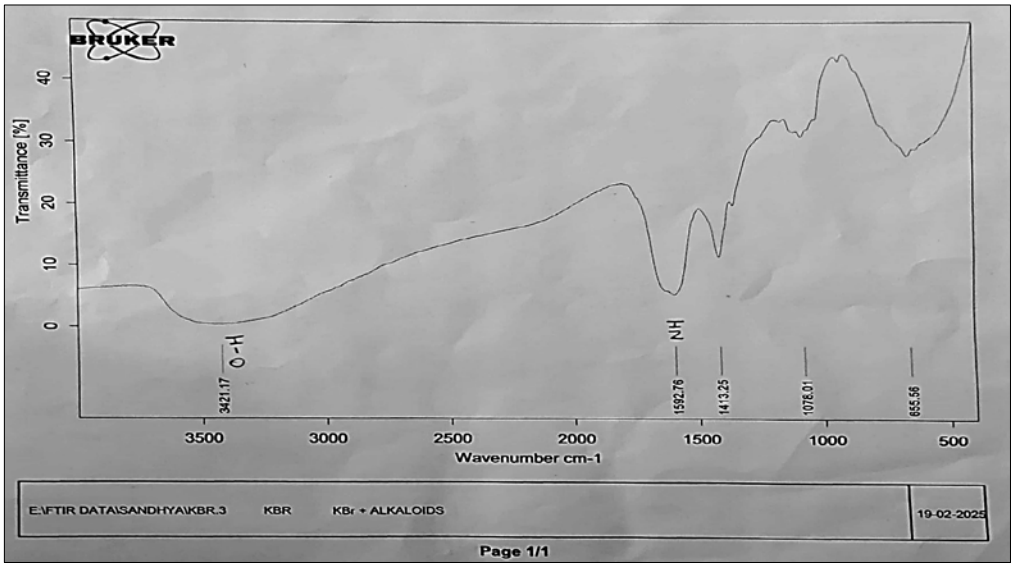

Fig 6: FTIR spectrum for alkaloids

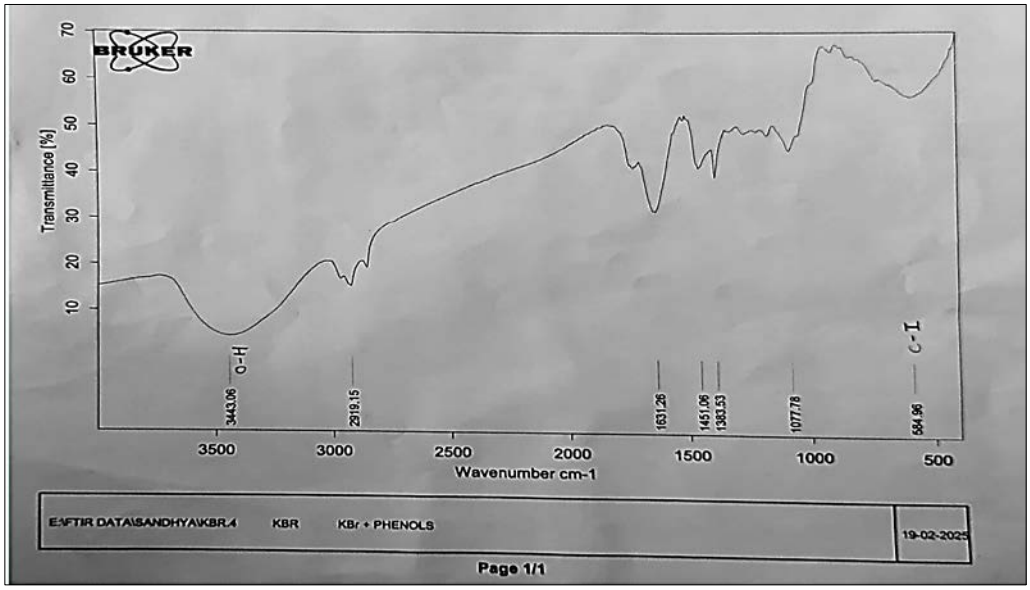

Fig 7: FTIR spectrum for phenols

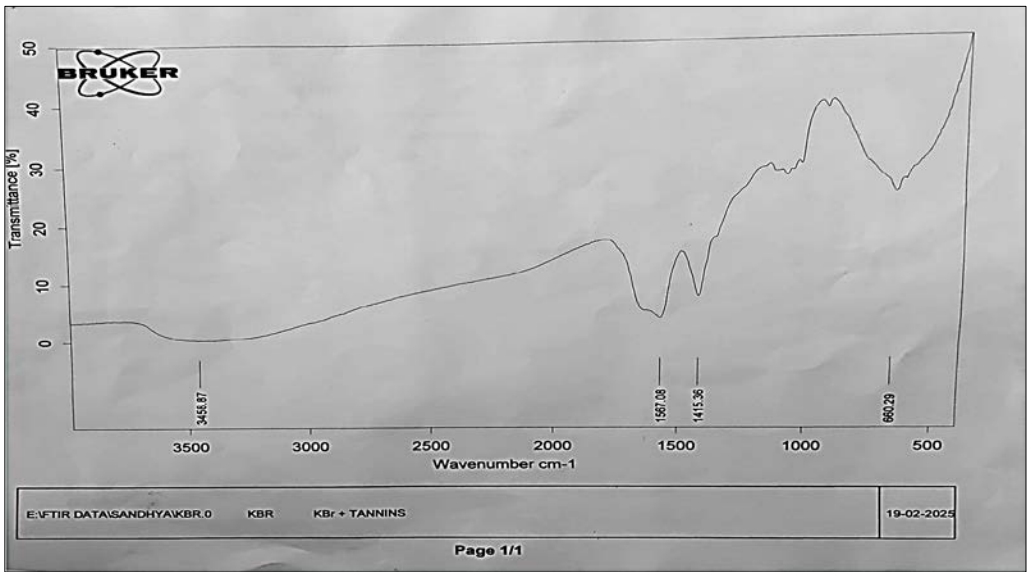

Fig 8: FTIR spectrum for tannins

Tables 6, 7, and 8 indicate the FTIR interpretation for alkaloids, phenols, and tannins.

Table 1: Standard qualitative procedures for phytochemical testing

Secondary Metabolites	Phytochemical Tests	Procedure
Alkaloids	Wagner's Test	2 ml of extract + a few drops of Wagner's reagent
Tannins	Ferric Chloride Test	3 ml of extract + 2 ml of 10% $FeCl_3$
Phenols	Ferric Chloride Test	Extract + 3-4 drops of 5% $FeCl_3$
Quinones	conc. HCl Acid Test	Extract + conc. HCl

Table 2: The solvent system used in the TLC method

Secondary metabolites	Solvent ratio
For Alkaloids	n-Propanol: Formic acid: water (20:3:2)
For Phenols	Ethyl acetate: n-Hexane (15:10)
For Tannins	Glacial acetic acid: water: n-Butanol (5:5:15)

Table 3: Phytochemical screening of *Murraya koenigii* extract

Secondary metabolites	Phytochemicals Tests	Observation	Interference
Alkaloids	Wagner's Test	Reddish-brown colored ppt	Alkaloids present
Tannins	Ferric Chloride Test	Dark blue-blackish colour	Tannins present
Phenols	Ferric Chloride Test	Deep blue colour	Phenols present
Quinones	conc. HCl Acid Test	Yellow ppt	Quinones absent

Table 4: The R_f (Retardation Factor) values for the separated phyto-constituents of *Murraya koenigii* extract

Phyto-constituents	R_f value
For Alkaloids	(Yellow-Green) 0.15
For Phenols	(Yellow-green) 0.17
For Tannins	(Green) 0.17

Table 5: The zone of inhibition of curry leaf extract, ethanol, and ciprofloxacin.

Sample in cavities/wells	Zone of inhibition (mm)	Interpretation of Antibacterial Activity
Ciprofloxacin drug - 100 µg/ml standard	35	Strong antibacterial effect
Curry leaves extract - 80 µg/ml sample	16	Moderate Activity
Ethanol - Solvent	1	Negative control

Table 6: Fourier Transform Infrared spectrum interpretation for Alkaloids

Absorption band (cm^{-1})	Vibration Type	Intensity	Functional Group
3421.17	O-H Stretch (Hydrogen Bonded)	Broad & Strong	Phenol or Alcohol
1592.76	N-H Bending or C=C stretching	Medium	Amine/Amide or Aromatic
1413.25	C-H Bending	Medium	Alkane or Aromatic Ring
1078.01	C-O Stretch	Medium	Ether or Alcohol
655.56	C-Br or C-I Stretch	Weak to Medium	Alkyl Halide (C-Br or C-I bond)

Table 7: Fourier Transform Infrared spectrum interpretation for Phenols

Absorption band (cm^{-1})	Vibration Type	Intensity	Functional Group
3443.06	O-H Stretch (Hydrogen Bonded)	Broad & Strong	Phenol or Alcohol
2919.15	C-H Stretch (Alkane)	Medium	Aliphatic C-H
1631.26	C=C stretch (Aromatic)	Medium to Strong	Aromatic Ring
1451.06	C-H Bending (Methyl or Methylene)	Medium	Alkane
1383.53	O-H Bending (Phenol) or C-H Deformation	Medium	Phenols or Alkanes
1077.78	C-O Stretch (Phenol or Ether)	Strong	Phenols or Ethers
564.96	C-Br Stretch	Weak to Medium	Alkyl Halide (C-Br bond)

Table 8: Fourier Transform Infrared Spectrum Interpretation for Tannins

Absorption band (cm^{-1})	Vibration Type	Intensity	Functional Group
3458.07	O-H Stretch (Hydrogen Bonded)	Broad & Strong	Phenol or Alcohol
1567.08	C=C Stretch (Aromatic)	Medium to Strong	Aromatic Ring
1415.36	C-H Bending (Methyl or Methylene)	Medium	Alkane or Aromatic Ring
660.29	C-Br or C-Cl Stretch	Weak to Medium	Alkyl Halide (C-Br or C-Cl bond)

Discussion

The present study demonstrated that the ethanolic extract of *Murraya koenigii* leaves contained a wide range of secondary metabolites, including alkaloids, tannins, and phenolic compounds, as confirmed by preliminary phytochemical screening. These phytochemicals are well recognized for their diverse pharmacological activities, thereby supporting the medicinal relevance of curry leaves

beyond their traditional culinary use. Antimicrobial evaluation of the extract revealed significant inhibitory activity against *E. coli*, highlighting its potential as a natural antimicrobial agent. The zone of inhibition for the ciprofloxacin drug, curry leaf extract, and ethanol is 35 mm, 16 mm, and 1 mm, respectively. The observed bioactivity may be attributed to the synergistic action of phenolic compounds and alkaloids, which are known to disrupt microbial cell structures and inhibit essential metabolic pathways. Such evidence strengthens the argument for considering *M. koenigii* as a candidate for developing alternative antimicrobial formulations in response to the growing problem of antibiotic resistance. The chromatographic profiling provided further resolution of the bioactive constituents. The R_f values for the separated secondary metabolites are 0.15 (alkaloids), 0.17 (phenols), and 0.17 (tannins). Thin Layer Chromatography (TLC) enabled the identification of an optimal solvent system, which facilitated effective separation. Column chromatography using this optimized system successfully isolated fractions enriched with secondary metabolites, thereby offering purified samples for further structural characterization. This step highlights the importance of solvent selection and chromatographic techniques in achieving reproducible phytochemical separation. Fourier Transform Infrared (FTIR) spectroscopy of the isolated fractions revealed multiple absorption bands corresponding to hydroxyl, amine, ether, amide, and alkyl halide groups, indicating the structural complexity of the extract. The presence of these functional groups suggests that phenolics, alkaloids, and halogenated compounds contribute to the observed pharmacological activities. Together, the integration of phytochemical, chromatographic, and spectroscopic analyses establishes *M. koenigii* as a rich source of bioactive compounds.

Conclusion

The study revealed that the *Murraya koenigii* extract was a rich source of secondary metabolites such as alkaloids, tannins, and phenols. *Murraya koenigii* exhibited antimicrobial activity against a wide range of microorganisms. The solvent systems used in TLC are cost-effective and have less volume consumption. The fractions of curry leaf extract collected through column chromatography were analyzed using Fourier Transform Infrared (FTIR) spectroscopy to identify functional groups. FTIR analysis confirmed the presence of ether, alcohol, amine, amide, and alkyl halide groups in the ethanolic extract of curry leaves. The study was further explored for integration with other analytical techniques and quality applications, with future prospects suggested in advanced research, clinical validation, and herbal formulation.

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Conflict of interest

The authors express no conflict of interest with anyone.

Abbreviations

FTIR: Fourier Transform Infrared Spectroscopy; TLC: Thin Layer Chromatography; RBF: Round Bottom flask; R_f: Retardation Factor; ZOI: Zone of Inhibition; Conc. HCl: Concentrated Hydrochloric acid; mm: Millimeters

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