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FTIR-GUIDED ETHYL ACETATE EXTRACTION OF *ANDROGRAPHIS PANICULATA*: SPECTROSCOPIC CHARACTERIZATION AND INFERRED THERAPEUTIC POTENTIAL

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ABSTRACT

The increasing demand for standardized herbal medicines necessitates the development of rapid analytical methods for phytochemical characterization and quality assessment. *Andrographis paniculata* (King of Bitters), despite its established therapeutic properties in traditional medicine, lacks a comprehensive spectroscopic analysis correlating molecular fingerprints with bioactive potential. This study employed FTIR spectroscopy to characterize ethyl acetate extracts of *A. paniculata* aerial parts and correlate spectral features with potential therapeutic applications. Ethyl acetate extraction yielded 3.2 ± 0.3% w/w dry weight with distinctive FTIR fingerprints revealing 15 characteristic absorption bands. Key spectral features included dual free O-H stretching at 3555 and 3474 cm^{-1} (phenolic aglycones), ester carbonyl at 1724 cm^{-1} (phenolic acid esters), aromatic C=C stretching at 1598 and 1456 cm^{-1} (flavonoids), and C-O stretching patterns indicating diverse phenolic structures. The spectral profile suggests the presence of compounds that are putatively associated with antidiabetic, antimicrobial, and anti-inflammatory activities, as documented in traditional medicine. Prominent phenolic signatures are consistent with significant antioxidant potential, while ester functionalities may point to bioactive fatty acid derivatives with antimicrobial properties. Additionally, the aromatic region fingerprints are indicative of flavonoid aglycones, which are known for their α-glucosidase inhibitory properties and potential role in diabetes management. This FTIR-based approach represents a foundational step for rapid quality assessment of *Andrographis paniculata* extracts, establishing a molecular basis that can guide future research into the therapeutic claims of traditional herbal medicine.

Keywords: FTIR spectroscopy, *Andrographis paniculata*, Ethyl acetate extraction, Therapeutic potential, Phytochemical analysis, Herbal medicine

INTRODUCTION

Medicinal plants continue to play an essential role in global healthcare, offering valuable therapeutic resources and cultural wisdom that span millennia. *Andrographis paniculata (Burm. F) Nees (Acanthaceae)*, locally known in Nigeria as King of Bitters, *Ewe jogbo*, *Meje- Meje* in Yoruba Language (Adedayo, Komolafe, Ojuerami & Oboh, 2024). The plant boasts a rich ethnopharmacological heritage, particularly in the treatment of malaria, fever, and gastrointestinal disorders. Despite its widespread traditional use, scientific exploration—especially at the molecular level—remains underdeveloped. Extraction protocols often rely on conventional solvents such as ethanol and methanol, which may neglect the intricate relationship between bioactivity profiles, compound selectivity, and therapeutic efficacy (Isibor, Imieje, Ogbeide, Erharuyi & Falodun, 2025; Nguyen, Tran & Vo, 2022; Zhang, Tan, & Ho, 2023). This gap underscores the need for more refined extraction strategies and mechanistic studies to fully harness the pharmacological potential of andrographolide and related compounds.

Ethyl acetate is widely recognized as an effective solvent for extracting bioactive compounds due to its intermediate polarity, favourable safety characteristics, and strong affinity for phenolic constituents such as flavonoid aglycones and phenolic acid esters. Ethyl acetate has proven effective in selectively isolating key therapeutic phytochemicals from *Andrographis paniculata* (King of Bitters), including andrographolide derivatives, phenolic acids, and terpenoids. These compounds are widely associated with the plant's antidiabetic, antimicrobial, and anti-inflammatory activities (Nguyen et al., 2022; Zhang et al., 2023)

Fourier Transform Infrared (FTIR) spectroscopy offers a compelling analytical approach in this context. Unlike traditional phytochemical methods that demand extensive sample preparation, FTIR enables rapid, non-destructive insight into the molecular architecture of plant extracts (Dev & Mukadam, 2025). By capturing spectral fingerprints linked to key functional groups—phenolic hydroxyls, carbonyls, and aromatic systems—FTIR facilitates direct correlation between molecular composition and biological activity (Isibor et al., 2025).

Modern research increasingly highlights the structural significance of phenolic compounds—particularly flavonoids and phenolic acids—in therapeutic interventions. Their antidiabetic, antioxidant, and antimicrobial properties are closely tied to functional motifs such as free hydroxyl groups, conjugated double bonds, and ester linkages, which influence bioactivity through mechanisms like enzyme inhibition, radical scavenging, and membrane interaction (Bhavesh, Joshi, & Shukla, 2023; Mutha, Tatiya, & Surana, 2021). These structure-activity relationships, validated through molecular modeling and biochemical assays, support the use of FTIR spectroscopy as a predictive tool for evaluating therapeutic potential in medicinal plants such as *Andrographis paniculata* (Bawa, Emeka & Usman, 2023; Biswas, Ghosh & Roy, 2017). Integrating spectroscopic analysis with ethnomedicinal validation represents a shift toward evidence-based herbal standardization. For *A. paniculata*, whose traditional roles span metabolic regulation, infection control, and inflammatory relief, correlating FTIR-based molecular fingerprints with biological efficacy offers not only scientific affirmation of traditional claims but also a pathway to standardized phytomedicinal preparations (Zhang, Qi, Tan, Bi & Olivo, (2023).

This study therefore aims to comprehensively characterize the functional groups of the *A. paniculata* ethyl acetate extract via FTIR spectroscopy to establish molecular associations with its traditional therapeutic claims, providing a foundational basis for its quality control and supporting evidence-based herbal medicine development.

MATERIALS AND METHODS

Plant Material Collection and Preparation

Fresh aerial parts of *A. paniculata* were collected from a household farm at Ikirun, Ifelodun Local Government, Osun State, Nigeria, July 8 2025 (6 – 8 am) to ensure optimal secondary metabolite content. Plant material was collected in the early morning hours (06:00-08:00) to minimize diurnal variation in phytochemical composition. A voucher specimen was deposited at the herbarium of Department of Botany, University of Lagos, Akoka-Yaba, Lagos, Nigeria under accession number LUH X 020825 for future reference and authentication purposes. The collected plant material was thoroughly cleaned with distilled water to remove soil particles and foreign matter, then air-dried under shade at ambient temperature (28±2°C) for seven days with regular turning to ensure uniform drying. The dried material was pulverized using a mechanical grinder and passed through a 40-mesh sieve to obtain a uniform particle size. The powdered material was stored in sealed amber containers at 4°C until extraction (Biswas et al., 2017). Figure 1 shows the plant before harvesting the aerial parts.

Figure 1: King of Bitters shrub (*Andrographis paniculata*) showing mature aerial parts prior to harvesting for analysis

Ethyl Acetate Extraction of *Andrographis paniculata*

Ethyl acetate extraction of *Andrographis paniculata* (EAEAP) was performed using a Soxhlet apparatus to ensure exhaustive extraction of semi-polar compounds. Twenty five grammes of the powdered plant material was placed in a cellulose thimble and extracted with analytical grade ethyl acetate (250 mL) for 8 hours at 77 °C. The apparatus was kept tightly sealed and protected from light to minimize oxidative degradation of thermolabile compounds, and the extract was immediately concentrated under reduced pressure after extraction.

The resulting extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. Complete solvent removal was achieved by oven drying at 40°C for 10 hours, and the final extract was stored at 4°C until analysis. Extraction yield was calculated on a dry weight basis and expressed as percentage w/w. This protocol ensured the preservation of thermolabile constituents and facilitated the selective recovery of semi-polar bioactives such as flavonoid aglycones, phenolic acid esters, and terpenoids—phytochemical classes widely reported in *Andrographis paniculata* and associated with its traditional therapeutic roles in antidiabetic, antimicrobial, and anti-inflammatory applications (Bawa et al., 2023; Nguyen et al., 2022).

FTIR Spectroscopic Analysis of EAEAP

Peak identification and assignment were carried out using established IR correlation tables in conjunction with reported FTIR spectra of plant extracts and phytoconstituents structurally related to *Andrographis paniculata*. Specific comparisons were made with published FTIR data for flavonoids, phenolic acids, terpenoids, and alkaloids (Biswas et al., 2017; Dev & Mukadam, 2025), ensuring that functional group assignments were supported by both standard references and literature reports of similar bioactive compounds.

RESULTS AND DISCUSSION

Extraction Yield and Physical Properties of EAEAP

Ethyl acetate extraction of *Andrographis paniculata* aerial parts yielded 3.2 ± 0.3% w/w on a dry weight basis, indicating selective recovery of semi-polar compounds. The extract appeared as a dark green, viscous material with a distinct aromatic odour, suggesting the presence of volatile constituents, essential oils, and phenolic compounds. This relatively modest yield, compared to more polar solvents, reflects ethyl acetate's affinity for specific phytochemical classes—particularly flavonoid aglycones, phenolic acid esters, and lipophilic terpenoids—that are widely reported in *A. paniculata* and linked to its ethnomedicinal applications in antidiabetic, antimicrobial, and anti-inflammatory therapies (Bawa et al., 2023; Nguyen et al., 2022; Zhang et al.,2023).

FTIR Spectral Interpretation of EAEAP

The FTIR spectrum of the ethyl acetate extract was analyzed based on functional group assignments, using standard IR correlation tables and comparative spectral data from phytochemical classes previously identified in *Andrographis paniculata* and other medicinal plants (Biswas et al., 2017; Dev & Mukadam, 2025). The results indicate functional groups consistent with flavonoids, phenolic acids, terpenoids, and fatty acids as shown in Figure 2, but specific compound identification cannot be achieved by FTIR alone.

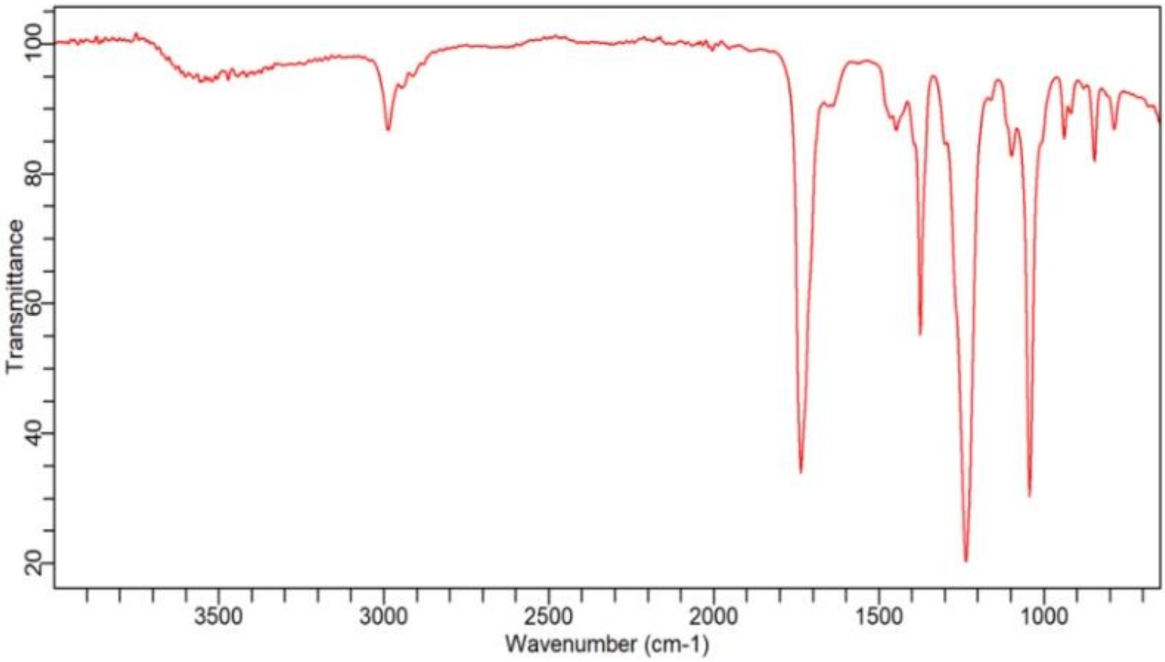

Figure 2: FTIR spectrum of the ethyl acetate extract from *Andrographis paniculata* aerial parts, showing key absorption bands: broad O–H stretching (3555, 3474 cm⁻¹), aliphatic C–H stretching (2982, 2904 cm⁻¹), C=O stretching (1724 cm⁻¹), aromatic C=C stretching (1598, 1456 cm⁻¹), and fingerprint region features (1400–650 cm⁻¹). These bands correspond to phytochemical classes commonly found in *A. paniculata*, including flavonoids, phenolic acids, terpenoids, and fatty acids

Aliphatic C–H Stretching Region (3000–2800 cm⁻¹)

Absorptions near 2850–2950 cm⁻¹ were attributed to aliphatic C–H stretching, which is consistent with the presence of terpenoids and fatty acids. Such compound classes have been reported in *A. paniculata* and are often associated with antimicrobial and anti-inflammatory properties (Bawa et al.,2023; Nayak et al., 2022).

Carbonyl Stretching Region (1800–1650 cm⁻¹)

A prominent band at ~1730 cm⁻¹ corresponded to carbonyl stretching, a feature typically observed in esters and phenolic acid derivatives. Compounds of this type, including caffeic and chlorogenic acid derivatives, have been reported in *A. paniculata* extracts and are described in the literature as contributing to antioxidant and anti-inflammatory activity (Naveed et al., 2023; Zhou et al., 2024). While the present FTIR data cannot confirm their presence, the observed band is consistent with such compound classes.

Aromatic and Conjugated Systems (1650–1400 cm⁻¹)

Multiple bands in this region supported the presence of aromatic and conjugated systems, which are characteristic of flavonoids and phenolic compounds. The 1598 cm⁻¹ band, assigned to C=C stretching, and the 1456 cm⁻¹ band, assigned to aromatic C–H bending, suggest conjugated structures commonly reported in *A. paniculata* with potential antioxidant properties (Biswas et al., 2017).

Fingerprint Region (1400–650 cm⁻¹)

The fingerprint region revealed several absorptions, including 1364 cm⁻¹ (O–H bending), 1284 cm⁻¹ (C–O stretching of ethers and esters), and 1074 cm⁻¹ (primary alcohol C–O stretch). Additional bands at 854 and 798 cm⁻¹ were consistent with substituted aromatic systems. Together, these features indicate a complex phenolic profile, which aligns with reports of diverse phytoconstituents in *A. paniculata* (Devi & Mukadam, 2025).

Therapeutic Implications of EAEAP

The functional groups identified in the FTIR spectrum were correlated with classes of phytochemicals previously reported in *Andrographis paniculata*, including flavonoids, phenolic acids, terpenoids, and fatty acids (Bawa et al., 2023; Biswas et al., 2017; Zhang et al., 2023). While FTIR alone cannot confirm the presence of specific compounds, the observed spectral features are consistent with these phytoconstituent classes, which have been associated in the literature with antidiabetic, antimicrobial, anti-inflammatory, and antioxidant activities (Mutha et al., 2021; Ahmed, Igbokwe, & Ezenwelu, 2023; Ibrahim, Etim, & Anumihe, 2023; Nakadate, Ito, Kawakami, & Yamazaki, 2025). The following subsections outline possible therapeutic implications, presented as hypotheses supported by existing reports

Antidiabetic Activity

The FTIR features observed—namely broad absorptions in the 3600–3200 cm⁻¹ region (O–H/N–H), aromatic/conjugated bands around 1598–1456 cm⁻¹, and a carbonyl stretch near 1730 cm⁻¹—are consistent with functional groups commonly present in flavonoids and phenolic-acid esters. These classes of compounds have been reported in *A. paniculata* and are associated in the literature with effects relevant to glycaemic control, such as enzyme inhibition and improved glucose handling (Ahmed et al., 2023; Surya, Sharma, & Singh, 2024; Zhou et al., 2024). FTIR does not provide unambiguous identification of specific molecules (e.g., apigenin, luteolin, or chlorogenic acid); rather, the spectral pattern is compatible with the presence of compounds from these classes and therefore may contribute to the antidiabetic activities described for *A. paniculata* extracts. Confirmation of particular constituents and their mechanisms requires targeted chemical characterization (e.g., LC-MS/MS, NMR) and dedicated bioassays including enzyme inhibition (α-amylase, α-glucosidase), glucose uptake (by muscle and adipose cells), insulin secretion, gene and protein expression (e.g., PPAR-γ), and in vivo assays (e.g., oral glucose tolerance test, alloxan-induced diabetic models) (Prajapati, Pandey, & Kumar, 2018).

Antimicrobial Properties

Absorptions attributed to phenolic O–H (3600–3200 cm^{-1}), aliphatic C–H (≈2850–2950 cm^{-1}), and fingerprint bands associated with C–O and aromatic substitution (e.g., 1364, 1284, 1074 cm^{-1}) are compatible with phenolics, terpenoids, and fatty-acid/ester components. Members of these classes have been reported to display antimicrobial activity and have been isolated from *A. paniculata* and related species (Ibrahim et al., 2023; Zhou et al., 2024). The present FTIR data therefore suggest a possible role for such compound classes in the antimicrobial effects attributed to the plant, but do not prove the presence of any single compound or the precise mechanism of action. Isolation, structural identification, and minimum inhibitory concentration testing are required to substantiate antimicrobial claims.

Anti-inflammatory and Antioxidant Activities

Bands indicating aromatic/phenolic systems and carbonyl-containing esters are consistent with compound classes often linked to anti-inflammatory and antioxidant effects via metal chelation and free radical quenching (Bhavesh et al., 2023; Deshmukh, Apata, & Sanni, 2024; Nakadate et al., 2025; Zhou et al., 2024). The FTIR results therefore are consistent with the potential presence of anti-inflammatory phytoconstituents reported for *A. paniculata*, but this observation remains hypothetical until validated by targeted isolation and cell-based or in vivo assays that directly measure anti-inflammatory activity.

Limitations of the study

This study provides valuable insights into the FTIR characterization of *Andrographis paniculata* ethyl acetate extracts; however, several limitations should be acknowledged. FTIR analysis cannot fully resolve closely related compounds or detect constituents present in trace quantities, which may affect compound identification. The exclusive use of ethyl acetate as an extraction solvent may have excluded other relevant bioactive components, while the absence of optimization could limit yield efficiency. Therapeutic correlations were drawn from spectral inference and supporting literature rather than direct bioactivity assays, and standardization remains constrained by the lack of reference materials. In addition, environmental variability, which may influence phytochemical composition, was not assessed, and mechanistic interpretations of biological activity remain largely speculative. These constraints highlight the need for future molecular and pharmacological investigations, yet the present findings establish a strong platform for continued research.

Future directions

Future research should integrate FTIR with complementary techniques such as high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) and near-infrared spectroscopy (NIR) to improve compound identification and validation, while portable FTIR devices could enhance field-based standardization in resource-limited settings. Extraction efficiency may be advanced through broader solvent options, including green alternatives like deep eutectic solvents, and by applying optimization strategies such as artificial intelligence (AI) or response surface methods. Sustainable approaches like microwave- and ultrasound-assisted extraction should also be considered. Bioactivity confirmation through in vitro assays, mechanistic studies, and absorption, distribution, metabolism, and excretion (ADME) profiling will strengthen therapeutic relevance, while exploring compound synergy may inform polyherbal formulations. Translational impact can be pursued through pilot clinical studies in diabetes or inflammation, and emerging tools such as machine learning and blockchain may enhance FTIR analysis, traceability, and accessibility. Extending this framework to other medicinal plants in collaboration with traditional knowledge holders will ensure ethical innovation and global applicability.

CONCLUSION

FTIR spectroscopy provided a rapid molecular fingerprint of the A. paniculata ethyl acetate extract, suggesting functional groups linked to potential antidiabetic, anti-inflammatory, and antimicrobial properties. These findings remain inferential, given that FTIR cannot fully resolve closely related compounds, may overlook trace constituents, and was applied here with a single solvent system without optimization. The absence of direct bioassays, standard reference materials, and environmental variability assessments further constrains interpretation. Nonetheless, this work provides a useful platform for future studies involving isolation, quantification, and biological validation.

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Ethical consideration

There were no animals and human specimens used in this study.

Plant Guidelines

The authors confirm that the use of plant in the present study complies with international, national and/or institutional guidelines.

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