Phytochemical Screening and FTIR Spectroscopic Characterisation of *Melastoma malabathricum* L. Leaf Extract: Preliminary Findings and Methodological Considerations for Extract Standardisation

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Abstract. *Melastoma malabathricum* L. (senduduk) is a Southeast Asian medicinal shrub whose leaves are rich in phenolic constituents, yet the chemical profiles of Indonesian accessions remain sparsely documented. Fourier-transform infrared (FTIR) spectroscopy is increasingly applied to fingerprint crude plant extracts, although sample preparation is seldom reported in detail. This study characterised a methanolic leaf extract from Palembang, South Sumatra, by qualitative phytochemical screening and evaluated the suitability of FTIR spectroscopy for this material. Dried leaf powder was macerated in methanol (1:10 w/v, 3 × 24 h) and concentrated under reduced pressure at 40 °C. Extraction yield was determined gravimetrically from three independent sub-batches. Screening for alkaloids, flavonoids, tannins, phenolics, saponins, steroids and triterpenoids was performed in triplicate, and infrared spectra were recorded on a Shimadzu spectrometer at 2 cm^{-1} resolution with 45 co-added scans. The extract was obtained in 10.0 ± 0.5% (w/w) yield. Screening indicated the presence of multiple phenolic-associated constituent classes — tannins, phenolic compounds, and flavonoids — alongside alkaloids, saponins, and steroids, consistently detected across replicates; however, triterpenoids were not detected. The infrared spectrum, however, was dominated by hydrogen-bonded hydroxyl (3313 cm^{-1}) and C–O (1021 cm^{-1}) vibrations, while carbonyl (1750–1600 cm^{-1}) and aromatic ring (1610 and 1515 cm^{-1}) absorptions were absent, despite unambiguous colorimetric evidence for phenolic constituents in the same material. This divergence is attributed to residual extraction solvent in material analysed without prior drying to constant mass. Therefore, the spectrum cannot be regarded as a chemical fingerprint, and verified solvent removal is a prerequisite for interpretable infrared analysis of crude plant extracts.

Keywords: Melastoma malabathricum, phytochemical screening, FTIR spectroscopy, extract standardization.

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1 Introduction

Oxidative stress is a recognised contributor to diabetes-associated complications, and there is ongoing interest in plant-derived bioactive compounds as potential adjunctive agents in this context [1], [2]. Rigorous chemical characterisation of candidate plant extracts, however, is a necessary prerequisite before any such biological potential can be meaningfully investigated.

Medicinal plants are important sources of structurally diverse secondary metabolites with antioxidant, anti-inflammatory, and metabolic activities. Melastoma malabathricum L. (*Melastomataceae*, locally known as senduduk) is traditionally used in Southeast Asia and has attracted pharmacological interest because its leaves contain flavonoids, tannins, saponins, phenolics, steroids/triterpenoids, glycosides, and other constituents [3], [4]. The compounds reported from the leaves or their fractions include quercetin, quercitrin, kaempferol derivatives, and ellagic acid [5], [6].

Prior studies have reported antioxidant- and antihyperglycemic-associated activities of senduduk leaf preparations [7], [8], which has motivated interest in this species for further pharmacological investigation. However, the chemical composition of a given extract can vary substantially with plant origin, extraction solvent, processing conditions, and analytical method; therefore, findings from one extract cannot be reliably extrapolated to another. Characterisation of the specific extract used in any subsequent study is therefore an essential prerequisite — which is the focus of the present work.

Qualitative phytochemical screening, based on colorimetric and precipitation reactions, provides a rapid and low-cost first indication of the major secondary-metabolite classes present in an extract and is widely accepted as an initial step in natural-product characterization. However, such assays are class-specific rather than compound-specific and provide no information on the molecular environment of the constituents [9], [10].

Fourier-transform infrared (FTIR) spectroscopy complements this limitation by generating a vibrational fingerprint of the extract as a whole. Absorption bands corresponding to hydroxyl (≈3400–3200 cm^{-1}), aliphatic C–H (≈2920–2850 cm^{-1}), carbonyl (≈1750–1600 cm^{-1}), aromatic C=C (≈1600–1450 cm^{-1}), and C–O (≈1260–1000 cm^{-1}) stretching allow inference of phenolic, flavonoid, and tannin-associated functional groups [11], [12]. FTIR is non-destructive, requires minimal sample preparation, and is increasingly applied to authentication, batch-to-batch comparison, and quality control of herbal materials. Used together, the two approaches provide orthogonal and mutually reinforcing evidence of extract composition. The reliability of such spectra, however, depends critically on sample presentation, as residual solvent or incomplete drying can dominate the absorption profile of a crude extract and obscure the contributions of its constituents.

Despite the growing pharmacological literature on M. malabathricum, most reports emphasise biological activity while providing limited characterisation of the specific extract tested, and the chemical profile of Indonesian accessions remains sparsely documented. The present work therefore characterises methanolic senduduk leaf extract from Palembang, South Sumatra, and concurrently evaluates the methodological re-

quirements that determine whether FTIR spectroscopy can serve as a reliable standardisation tool for crude extracts of this type. Together, these provide a documented chemical reference and a preliminary extraction protocol whose repeatability and reproducibility remain to be formally validated in subsequent work.

This study aimed to characterise methanolic senduduk leaf extract of Palembang origin through qualitative phytochemical screening, and to evaluate the suitability of FTIR spectroscopy as a complementary fingerprinting tool for this material. Leaves were extracted by maceration in methanol, screened for alkaloids, flavonoids, tannins, saponins, phenolics, and steroids/triterpenoids, and analysed by FTIR. A secondary aim was to examine the influence of sample preparation on the interpretability of the infrared spectra of crude plant extracts, and to outline a preliminary characterisation protocol, noting that formal assessment of its reproducibility (e.g., across extraction batches, instruments, or operators) was beyond the scope of the present study. The study does not claim direct nephroprotective efficacy.

2 Material and Methods

This was an experimental laboratory study with a descriptive-exploratory design. Two complementary analytical approaches were applied to a single extract, including qualitative analysis, comprising colorimetric and precipitation-based phytochemical screening to detect classes of secondary metabolites, and quantitative analysis, comprising gravimetric determination of extraction yield and measurement of FTIR absorption bands expressed as wavenumbers (cm^{-1}) and percentage transmittance. No comparison group or intervention was involved, and no inferential hypotheses were tested.

2.1 Plant material

Fresh leaves of senduduk were collected from Palembang, South Sumatra, Indonesia. Leaves were collected in July 2026 from mature and disease-free plants. The specimen was authenticated at Fakultas Sains dan Teknologi Universitas PGRI Palembang, and a voucher specimen No.206/E.12/FST/UPGRI/2026 was obtained. Leaves were processed within 2 hours of collection, air-dried at 25–30 °C and 60–70% relative humidity for approximately 3 days until constant weight, then ground to a coarse powder, and stored in an airtight, light-protected container at room temperature for no longer than 3 days prior to extraction.

2.2 Extract Preparation

Bulk extraction was performed on 500 g of dried leaf powder to provide material for all subsequent analyses. The powder was macerated in methanol (1:10 w/v, 5 L per cycle) for 3 × 24 h at room temperature with intermittent stirring, with the solvent renewed at the end of each 24 h cycle. The combined filtrates were filtered through Whatman No. 1 paper and concentrated using a rotary evaporator at 40 °C under reduced

pressure. The concentrated extract was stored at 4 °C in a sealed, light-protected container and used for phytochemical screening and FTIR analysis within 1 days of preparation. The extraction yield was determined separately using three independent 50 g sub-batches processed under identical conditions and calculated gravimetrically.

2.3 Qualitative Phytochemical Screening

Screening was performed in triplicate on three independently prepared aliquots taken from the single bulk methanolic extract. A class was reported as present only when consistently detected in all three replicates. Alkaloids were detected using Dragendorff reagents; flavonoids using the Willstätter cyanidin test (Mg turnings + conc. HCl); tannins and phenolics with 5% $FeCl_3$; saponins using the persistent-froth test; and steroids and triterpenoids using the Liebermann–Burchard reaction. Results were recorded as detect (+) or not detect (−) based on the appearance of the characteristic precipitate or colour change relative to a reagent blank, and a class was reported as present only when detected consistently in all three replicates.

2.4 Fourier-transform Infrared Analysis

Infrared spectra were acquired on a Shimadzu FTIR spectrometer (Shimadzu Corporation, Kyoto, Japan) fitted with a single-reflection attenuated total reflectance (ATR) accessory equipped with a diamond crystal. The concentrated extract was applied directly to the crystal surface without further drying, and uniform contact was ensured using an integrated pressure clamp. Spectra were recorded over 4500–350 cm^{-1} at a resolution of 2 cm^{-1} with 45 co-added scans, against a background of the clean, empty crystal collected immediately beforehand under identical conditions. The crystal was cleaned with ethanol and dried between measurements. Measurements were performed in triplicate by independently applying separate aliquots of the same bulk extract to the ATR crystal (technical replicates), each preceded by a freshly collected background spectrum.

2.5 Data Analysis

Screening results were tabulated descriptively. FTIR spectra were baseline-corrected using LabSolutions IR software, and the absorption maxima were identified and tabulated with their wavenumbers and transmittance values. Spectra were smoothed prior to peak identification using the Savitzky–Golay algorithm implemented in LabSolutions IR; no other spectral processing was applied. Functional groups were assigned by comparison with standard infrared correlation tables and previously published spectra of phenolic-rich plant extracts. Findings from both approaches were then integrated to construct a composite chemical profile of the extract. Although FTIR measurements were performed in triplicate, band positions and intensities from individual replicate spectra were not retained for quantitative comparison; the spectrum presented represents a single representative measurement, and inter-replicate variability was assessed only qualitatively by visual comparison of the three spectra.

3 Results

3.1 Extraction Yield

The mean extraction yield, determined from three independent 50 g sub-batches, was 10.0 ± 0.5%. Bulk maceration of 500 g of dried leaf powder yielded 48.4 g of dark greenish-brown, viscous crude extract with a characteristic astringent odour, which was used for all subsequent analyses.

3.2 Preliminary Phytochemical Profile

Qualitative screening of the crude methanol extract indicated the presence of a chemically diverse range of constituent classes, including several associated with phenolic compounds (Table 1), and the relative abundance of these classes cannot be inferred from qualitative testing. Addition of ferric chloride produced an immediate blackish-green colouration, indicating the presence of tannins fraction; a separate aliquot treated with the same reagent gave a green-to-red transition, confirming the general presence of phenolic hydroxyl functionality. The Willstätter cyanidin reaction, performed with magnesium turnings and concentrated hydrochloric acid, generated a yellowish-red colouration consistent with the presence of flavonoid-type constituents (tentatively suggestive of a flavone/flavonol-type structure).

Alkaloids were indicated by the formation of an orange turbid precipitate with Dragendorff's reagent. Vigorous agitation of an aqueous suspension of the extract produced a persistent froth of 1 cm that remained stable for 15 min. In the Liebermann-Burchard reaction the extract developed a violet colouration, indicating a steroidal nucleus. The reddish-brown interfacial ring diagnostic of triterpenoids was not observed.

Table 1. Extraction conditions and preliminary qualitative phytochemical profile of the crude methanol extract of senduduk leaves.

Parameter	Reagent and procedure	Observation	Inference
Extraction	Methanol; maceration, 1:10 (w/v), 3 × 24 h, 25 ± 2 °C	10.0 ± 0.5% (w/w) of dry plant material	Crude methanol extract obtained; dark green, viscous
Tannins	$FeCl_3$ (5%, w/v)	Blackish-green colouration	Tannins present (+)
Phenolic constituents	$FeCl_3$ (5%, w/v)	Green shifting to red	Phenolic hydroxyl groups present (+)
Flavonoids	Willstätter cyanidin test (Mg turnings + conc. HCl)	Yellowish-red colouration	Flavonoids present (+)

Alkaloids	Dragendorff's reagent	Orange turbid precipitate	Alkaloids present (+)
Saponins	Distilled water, heated, vigorously shaken for 30 s	Persistent froth 1 cm, stable for 15 min	Saponins present (+)
Steroids	Liebermann-Burchard (Ac_2O, conc. H_2SO_4 and $CHCl_3$ were used as solvents).	Violet colouration	Steroidal nucleus present (+)
Triterpenoids	Liebermann-Burchard (as above)	No reddish-brown interfacial ring	Not detected (−) under the present conditions

(+) detected; (−) not detected

3.3 Fourier-transform Infrared Spectroscopic Profiling

The FTIR spectrum of the M. malabathricum L. leaf extract is presented in Figure 1, and the observed band positions with their tentative assignments are summarised in Table 2. As the sample represents a complex mixture of secondary metabolites rather than a purified constituent, the spectrum is interpreted as a composite profile in which individual bands reflect the dominant functional groups of the mixture rather than a single molecular species.

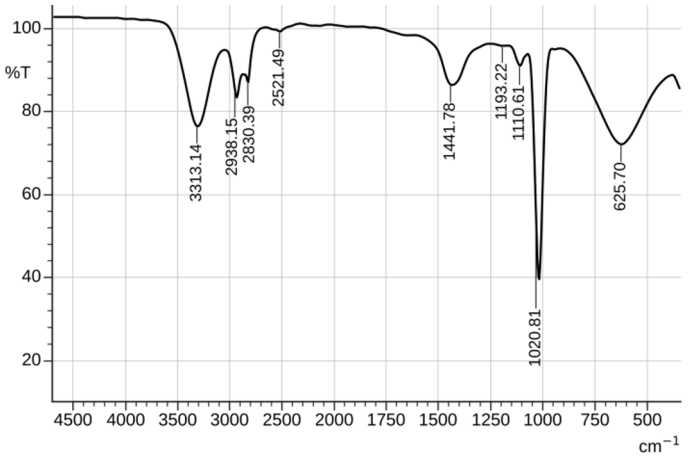


Fig. 1. FTIR spectrum of methanolic *Melastoma malabathricum* L. leaf extract recorded in ATR mode over 4500–350 cm^{-1} at 2 cm^{-1} resolution with 45 co-added scans. Spectra were smoothed using the Savitzky–Golay algorithm.

The profile is dominated by a strong, markedly broadened absorption centered at 3313 cm^{-1}, attributable to O–H stretching vibrations. The pronounced red shift relative to the free hydroxyl position (≈3650 cm^{-1}), together with the width of the envelope, indicates that the hydroxyl groups are extensively engaged in intermolecular hydrogen bonding rather than existing as isolated oscillators.

In the C–H stretching region, two resolved components appeared at 2938 and 2830 cm^{-1}, assigned to the asymmetric and symmetric stretching of aliphatic C–H bonds, respectively. The comparatively low frequency of the latter is characteristic of C–H oscillators attached to oxygen-bearing carbon atoms and is consistent with C–H oscillators adjacent to oxygen-bearing carbon atoms. Assignment to a specific moiety, such as a hydroxymethyl or methoxy group, cannot be established from this band alone and would require corroboration by complementary techniques. A weak feature at 2521 cm^{-1} was tentatively assigned to an overtone–combination band arising from O–H bending and librational modes.

Within the fingerprint region, the band at 1441 cm^{-1} is ascribed to asymmetric methyl deformation overlapping with in-plane O–H bending, while the weaker feature at 1193 cm^{-1} may arise from coupled C–O–H bending and methyl rocking. The most intense absorption, at 1021 cm^{-1} with a shoulder at 1110 cm^{-1}, is assigned to C–O stretching vibrations broadly associated with alcohol, ether, or carbohydrate-type functionality. Distinguishing a specific linkage type, such as a glycosidic bond, would require confirmation by complementary analytical techniques. The broad medium-intensity band at 626 cm^{-1} corresponds to the out-of-plane torsional (librational) motion of hydrogen-bonded hydroxyl groups.

Notably, no absorptions were detected in the regions characteristic of carbonyl (1750–1600 cm^{-1}) or aromatic ring (1610 and 1515 cm^{-1}) vibrations. Given that the leaves of this species are reported to contain hydrolysable tannins, flavonoids and their glycosides, all of which possess strong carbonyl and aromatic chromophores, the absence of these bands indicates that the recorded profile is not representative of the polyphenolic fraction of the extract.

Taken together, the spectral data indicate that the analysed material is dominated by hydrogen-bonded, hydroxyl- and C–O-rich components. Definitive assignment of the phytoconstituents cannot be established from vibrational data alone and requires corroboration by chromatographic and mass spectrometric analysis.

Table 2. FTIR absorption bands of methanolic senduduk leaf extract and their vibrational assignments

Observed band (cm^{-1})	Intensity/shape	Assigment
3313	Strong, very broad	ν(O–H), hydrogen-bonded hydroxyl
2938	Medium	ν_as(C–H), CH_3/CH_2
2830	Medium	ν_s(C–H), C–H adjacent to an oxygen-bearing carbon (generic)
2521	Very weak	Overtone/combination band of the O–H bending–librational modes

1441	Medium	δ_as(CH_3) overlapped with in-plane δ(O–H)
1193	Weak–medium	δ(C–O–H) / CH_3 rocking
1110	Medium	ν(C–O), coupled C–C–O
1020	Very strong	ν(C–O) stretching, consistent with alcohol and/or ether functionality (generic)
626	Broad, medium	Out-of-plane O–H torsion (libration)

4 Discussion

4.1 Phytochemical Screening Findings

Qualitative screening indicated the presence of several phenolic-associated and other constituent classes — tannins, phenolic compounds, and flavonoids, alongside alkaloids, saponins, and steroids — consistently detected across replicates, whereas triterpenoids were not detected under the conditions employed (Table 1). This pattern is broadly compatible with previous phytochemical reports on *M. malabathricum*, which described leaves containing flavonoids, tannins, saponins, and steroidal/triterpenoid constituents [3, 4]. However, as these assays rely on class-specific colorimetric and precipitation reactions, they indicate the presence or absence of broad metabolite classes only and do not permit inference of specific compound identity, relative abundance, or concentration.

4.2 FTIR Spectral Results

The FTIR profile obtained in the present study is characterised by three dominant features, including an intense and strongly broadened O–H stretching envelope at 3313 cm^{-1}, a resolved aliphatic C–H doublet at 2938 and 2830 cm^{-1}, and a very intense C–O stretching band at 1020 cm^{-1}. The magnitude of the red shift of the hydroxyl envelope relative to the free O–H position indicates that essentially all hydroxyl groups within the analysed material participate in intermolecular hydrogen bonding. Such extensive band broadening is typical of hydroxyl-rich systems in the condensed phase, where a distribution of hydrogen-bond geometries produces an inhomogeneously broadened envelope rather than discrete transitions [14].

Of greater interpretive significance, however, is the pattern of bands that were not observed. The leaves of M. malabathricum have been repeatedly shown to be rich in polyphenolic constituents. Successive extraction of the dried leaves yielded triterpene α-amyrin, amides auranamide and patriscabratine, and flavonoids quercitrin, quercetin, and kaempferol-3-O-(2″,6″-di-O-p-trans-coumaroyl)-β-glucoside [15], while the acetone extract afforded dimeric hydrolysable tannins (malabathrins B–D) together with monomeric galloyl glucosides, strictinin, casuarictin, and pedunculagin [3]. Every one of these structural classes possesses chromophores that absorb strongly in the mid-infrared: galloyl and coumaroyl esters give rise to C=O stretching near 1700 cm^{-1}, the flavonoid chromone carbonyl absorbs near 1650 cm^{-1}, and aromatic ring stretching modes appear consistently at approximately 1610 and 1515 cm^{-1}. The complete absence

of absorption in these regions is therefore inconsistent with a spectrum arising predominantly from the polyphenolic fraction of the extract. It should be emphasised that these specific compounds are drawn from prior phytochemical isolations of M. malabathricum reported in the literature [3], [18] and have not been identified or confirmed in the present extract. The FTIR data obtained here permit inference of functional-group classes only and cannot establish the presence of specific compounds such as quercetin, quercitrin, or kaempferol derivatives.

Some studies on senduduk leaf extracts emphasise a dominant hydrolysable-tannin fraction, particularly ellagitannins and ellagic acid derivatives [13]. Such compounds would be expected to produce a pronounced ester carbonyl absorption near 1730–1710 cm^{-1} together with intense aromatic C–O bands. The complete absence of these absorptions in the present spectrum, noted above, suggests that — if present at all — this fraction did not dominate the infrared profile of the analysed material, consistent with the broader interpretation that residual solvent obscured the contributions of the phytoconstituents (see below). Such variation is unsurprising: extraction solvent, plant maturity, geographic origin, and drying conditions all substantially influence the polyphenolic composition of senduduk [3], [7], reinforcing the argument advanced in the Introduction that each experimental extract requires its own characterisation.

4.3 Residual Solvent Effects

Several factors may account for this discrepancy. The most parsimonious explanation is the persistence of residual extraction solvent within the analysed material. The band positions recorded here, that are 3313, 2938, 2830, 1442, 1111, 1021 and 626 cm^{-1}, correspond closely to the reported vibrational frequencies of short-chain aliphatic alcohols, in which the symmetric C–H stretch is characteristically depressed below 2850 cm^{-1} by the adjacent oxygen atom and the C–O stretch dominates the fingerprint region [16]. In the present work, the extract was applied to the ATR crystal without prior drying to constant mass, and it is therefore probable that the solvent contribution masked the weaker absorptions of the dissolved phytoconstituents.

This interpretation is reinforced by the sampling geometry of the attenuated total reflectance. Because the evanescent field penetrates only one to two micrometres beyond the crystal surface, ATR measurements are strongly weighted towards the material in immediate contact with the crystal. In a liquid or semi-liquid sample, the mobile solvent phase preferentially occupies this interfacial region; therefore, minor solutes contribute disproportionately little to the resulting spectrum. A secondary consideration is the spectral processing applied: smoothing improves the visual appearance of the baseline but simultaneously attenuates weak, narrow features, and low-intensity aromatic bands are particularly vulnerable to suppression.

4.4 Methodological Limitations

These observations have broader significance for phytochemical literature: FTIR spectra of crude plant extracts are frequently reported as evidence for the presence of particular functional groups; however, sample preparation is often described only briefly,

and verification that the extraction solvent has been removed is rarely documented. The present results demonstrate that, in the absence of such verification, an infrared spectrum may reflect the solvent matrix rather than the phytoconstituents of interest, and that functional-group assignments derived from it may be substantially misleading. It should be noted that the residual-solvent explanation advanced above was inferred from comparison with literature spectra of short-chain aliphatic alcohols, rather than experimentally verified within the present study; no dried (constant-mass) aliquot or solvent-only blank spectrum was analysed for direct comparison here, and repeatability, batch-to-batch reproducibility, and spectral similarity were not formally assessed.

Accordingly, the vibrational data cannot be regarded as characterising the phytochemical composition of the extract: no inference concerning the presence, absence, or relative abundance of specific metabolite classes can be drawn from these data, and the assignments in Table 2 remain tentative. Therefore present the following as methodological recommendations for future FTIR characterisation of crude extracts, rather than procedures validated in the present work: that drying to constant mass be confirmed gravimetrically prior to analysis, that a solvent-only blank be recorded on the same crystal and reported as supporting information, that full spectral-processing parameters (e.g., smoothing window size and polynomial order) be explicitly reported, and that repeatability and batch-to-batch reproducibility be formally assessed where FTIR is proposed as a standardisation tool.

4.5 Implications for Future Research

Confirmation of the phytochemical profile of the present extract requires re-analysis of lyophilised material, corroborated by chromatographic separation and mass spectrometric identification; which is currently in progress. More broadly, establishing FTIR as a validated standardisation tool for this material will require formal assessment of inter-day and inter-instrument repeatability, extraction batch-to-batch reproducibility, and quantitative spectral similarity metrics, none of which were addressed in the present preliminary characterisation. Any subsequent evaluation of the pharmacological potential of this extract should be based on this validated chemical and analytical foundation, rather than directly extrapolated from bioactivity data reported for other accessions or preparations in the literature.

5 Conclusion

Methanolic maceration of senduduk leaves from Palembang, South Sumatra, yielded 10.0 ± 0.5% (w/w) crude extract. Qualitative screening indicated the presence of multiple phenolic-associated and other constituent classes, including tannins, phenolic compounds, flavonoids, saponins, steroids, and alkaloids, consistently detected across replicates, whereas triterpenoids were not detected under the conditions employed.

The FTIR spectrum did not corroborate this profile. Hydrogen-bonded hydroxyl and C–O vibrations dominated, while carbonyl and aromatic ring absorptions were absent despite unambiguous colorimetric evidence for phenolic constituents in the identical

material. This discrepancy is most plausibly attributed — on the basis of comparison with literature spectra of residual solvent, rather than direct experimental confirmation within this study — to residual extraction solvent, the extract having been analysed without prior drying to constant mass, compounded by the surface-weighted sampling geometry of attenuated total reflectance. The vibrational data therefore cannot be regarded as a chemical fingerprint, and the assignments in Table 2 remain provisional.

These observations suggest — based on spectral comparison with literature data rather than direct experimental verification — that infrared spectra of crude plant extracts may reflect the solvent matrix rather than the phytoconstituents when solvent removal is not verified gravimetrically. Definitive characterisation of this accession requires reanalysis of lyophilised material supported by LC-MS profiling, which will provide the standardised chemical basis required together with formal assessment of FTIR repeatability, reproducibility, and batch-to-batch spectral similarity, before this extract can be considered for further pharmacological investigation.

Disclosure of Interests. The authors declare no conflicts of interest.

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