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Original Research Article

Phytochemical Profiling of Limau Kuit (*Citrus jambhiri* Lush.) using FTIR Spectroscopy and Multivariate Analysis

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ABSTRACT

Citrus jambhiri Lush., locally known as Limau Kuit, is an indigenous citrus species from South Kalimantan, Indonesia, traditionally used by the Banjar community for food and medicinal purposes. This study aimed to characterize the phytochemical composition of various parts (leaves, fruit peel, fruit pulp, and fruit juice) of *C. jambhiri* using Fourier Transform Infrared (FTIR) spectroscopy combined with principal component analysis (PCA). Extractions were performed using three solvents of varying polarity: n-hexane, ethyl acetate, and ethanol. The phytochemical profile of the extracts was characterized by Fourier Transform Infrared (FTIR) spectroscopy followed by multivariate data analysis with chemometric techniques. FTIR spectra revealed the presence of key functional groups such as O–H, C–H, C=O, and C=C, suggesting the existence of secondary metabolites, including flavonoids, terpenoids, and saponins. Chemometric analysis using principal component analysis (PCA) and cluster analysis showed that solvent polarity had a greater impact on chemical composition than plant part, with clear clustering observed among extracts prepared using the same solvent. Juice extracts formed a distinct cluster, indicating the presence of unique compounds compared to other parts. These findings underscore the importance of solvent selection in targeting specific bioactive constituents from *C. jambhiri*. The study provides a scientific basis for the potential development of *C. jambhiri*-based functional food ingredients and herbal formulations and highlights the value of FTIR–PCA as a rapid, non-destructive method for phytochemical profiling.

Keywords: Chemometrics, *Citrus jambhiri* Lush., Fourier Transform Infrared Spectroscopy, Principal Component Analysis, Secondary Metabolites

Introduction

Indonesia is renowned for its remarkable biodiversity, particularly its wide array of native medicinal plants, which hold significant potential for advancing healthcare.[1,2] Among these, is Limau Kuit - a local citrus fruit traditionally used by the Banjar community in South Kalimantan.[3,4] Although, many previous studies referred to Limau Kuit as *Citrus amblycarpa*, recent morphological characterizations and taxonomic studies have confirmed that the correct scientific name is *Citrus jambhiri* Lush.[5] The taxonomic confusion may have arisen from morphological similarities between Limau Kuit and other citrus species, such as jeruk limo (*Citrus amblycarpa*), leading to misidentifications in earlier publications. Traditionally, *Citrus jambhiri* Lush has been widely used as a culinary ingredient, particularly for its aromatic and sour juice.

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This species, considered an exotic fruit, has become a culinary icon of South Kalimantan and thrives in lowland regions across Banjar, Barito Kuala, and neighboring areas. The plant typically bears fruit year-round, with peak production occurring during the rainy season, when high rainfall promotes the growth of more abundant and fresh fruit. *Citrus jambhiri* Lush plays a vital role in the lives of the Banjar people, serving not only as a culinary spice that enhances the flavor of traditional dishes such as soto Banjar and sambal, but also as a component of herbal remedies believed to aid digestion, relieve coughs, and boost physical endurance. Its widespread availability in traditional markets and household gardens underscores its integral role in both the cultural and health practices of the local community.

Beyond its culinary and ethnomedicinal role, several studies have highlighted the potential pharmacological properties of *Citrus jambhiri* Lush, including antioxidant, anti-aging, anti-inflammatory, and anti-diabetic effects. However, comprehensive investigations into its chemical profile remain limited, particularly concerning variations arising from different solvent-based extraction methods.

Although, research on the biochemical composition of *Citrus jambhiri* Lush has been conducted, it focused mainly on specific plant parts, and was primarily oriented toward industrial applications. Nevertheless, comprehensive profiling of its chemical constituents using advanced spectroscopic and chemometric techniques remains limited. This gap necessitates the need for further investigation into its bioactive compounds, which may reveal additional therapeutic properties.

Expanding such research would not only enhance the culinary value of *Citrus jambhiri* Lush but also unlock new opportunities for its application in healthcare, thereby contributing further to Indonesia's rich tradition of plant-based medicine.

Widely utilized by the Banjar people, *Citrus jambhiri* Lush offers both culinary versatility and considerable ethnomedicinal value, reflecting its deep cultural significance. Traditionally, this plant has been used in various healing practices due to its bioactive compounds with recognized health benefits. Preliminary studies have identified several secondary metabolites in *Citrus jambhiri* Lush, including tannins, flavonoids, saponins, and steroids, which exhibit larvicidal properties.[6] However, comprehensive characterization of these compounds, particularly with advanced analytical techniques such as Fourier Transform Infrared (FTIR) and chemometric analysis remains limited. Such studies are essential for understanding the full potential of these bioactive compounds and for uncovering additional medicinal benefits that may contribute to both traditional and modern healthcare.

FTIR spectroscopy offers a rapid, straightforward, and non-destructive method for analyzing the chemical composition of botanical specimens.[7,8] FTIR detects functional groups within molecules, enabling a comprehensive assessment of chemical constituents.[9,10] When combined with chemometric techniques such as principal component analysis (PCA), FTIR can elucidate diverse chemical constituents, and differentiate among specimens based on their molecular characteristics.[11–13] In this study, the combined application of FTIR and PCA is expected to play a crucial role in analyzing the chemical composition of different parts of *Citrus jambhiri* Lush, including its leaves, peel, pulp, and juice. Samples were extracted with three solvents; n-hexane, ethyl acetate, and ethanol, and extracts were analyzed to identify and compare the unique chemical characteristics of each plant part. This analysis is expected to provide valuable insights into the bioactive potential of *Citrus jambhiri* Lush.

The three selected solvents; n-hexane, ethyl acetate, and ethanol were chosen for their differences in polarities, which influence their ability to extract specific chemical compounds. n-Hexane, a non-polar solvent, is particularly effective in extracting hydrophobic compounds, while ethanol, a polar solvent, is more suitable for extracting hydrophilic compounds. Ethyl acetate, with intermediate polarity, offers a balanced extraction capability, enabling the isolation of both polar and non-polar compounds.[14] Consequently, this research aimed to elucidate the relationship between the types of solvents used and the resulting chemical composition of *Citrus jambhiri* Lush, and to examine how these variations may correlate with the potential biological activities of the extracts.

To date, no scientific study has specifically examined the influence of solvent polarity on the chemical composition of *Citrus jambhiri* Lush. Comprehensive comparisons of extracts from different plant parts using various solvents are still limited. This study offers a valuable preliminary insights into the plant's chemical constituents, that may inform future investigations into its biological activities and potential phytomedicinal applications. The results are expected to enhance the understanding of the chemical characteristics of *Citrus jambhiri* Lush and support its future application in the medical, pharmaceutical, and functional food industries. Furthermore, the outcome if this study would contributes to the scientific exploration of Indonesia's rich biodiversity and its potential to advance human health.

Materials and Methods

Plant materials

Citrus jambhiri Lush leaves, fruit peels, juice, and pulp were collected on 13 December 2023 from Astambul Sub-district, Banjar Regency, South Kalimantan Province, Indonesia (coordinates: 3°21'30.4"S, 114°53'46.1"E) (Figure 1). The plant specimen (*Citrus jambhiri* Lush) was identified by Dr. apt. Asni Amin, and apt. Abd. Malik, at the Laboratory of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Muslim University of Indonesia (UMI), Makassar, Indonesia. A herbarium specimen with voucher number 0158/C/UD-FF/UMI/II/2025 was deposited at the Herbarium of the Laboratory of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, UMI, Makassar, Indonesia.

Figure 1: Map of the sample collection site

Extraction of plant materials

The refined powder of the leaves, fruit peels, fruit juice, and fruit pulp of *Citrus jambhiri* Lush were extracted sequentially by maceration at room temperature (~28℃) using n-hexane (non-polar) for 1 day, followed by 70% ethanol (polar) for 3 days, with constant stirring with a mechanical stirrer to ensure thorough extraction.

For each extraction step, a material-to-solvent ratio of 1:10 (w/v) was applied, in this case, 125 g each of the powdered plant material was extracted with 1.25 L of solvent.

Dried fruit juice was obtained by squeezing approximately 250 fresh limes. The resulting juice was freeze-dried to yield 244 g of dried sample. This dried juice was then extracted sequentially by maceration in ethyl acetate (semi-polar) and subsequently with 70% ethanol (polar), applying the same material-to-solvent ratio of 1:10 (w/v).

Each extraction stage was performed once. The extracts were filtered, and the solvents were removed under reduced pressure using a rotary evaporator (Rotavapor® R-300, Buchi Corporation, USA) at 35℃ for n-hexane extracts and 40℃ for 70% ethanol extracts to obtain concentrated extracts. The extraction protocol was adapted and modified from the procedures described by Retnosari *et al*. (2023).[15]

The extracts obtained were coded as follows: P1 (ethyl acetate extract of *Citrus jambhiri* Lush. juice), P2 (ethanol extract of *Citrus jambhiri* Lush juice), D1 (n-hexane extract of *Citrus jambhiri* Lush leaves), D2 (ethanol extract of *Citrus jambhiri* Lush leaves), A1 (n-hexane extract of *Citrus jambhiri* Lush fruit pulp), A2 (ethanol extract of *Citrus jambhiri* Lush fruit pulp), K1 (n-hexane extract of *Citrus jambhiri* Lush fruit peel), and K2 (ethanol extract of *Citrus jambhiri* Lush fruit peel).

Data analysis

Phytochemical analysis using FTIR

Phytochemical tests using the Fourier Transform Infrared (FTIR) method were conducted in the mid-infrared region (4000–400 cm^{-1}) using a Shimadzu IRPrestige-21 FTIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan) equipped with a deuterated triglyceride sulfate (DTGS) detector. Spectral data acquisition and processing were performed using IRsolution software (Shimadzu Corporation, Kyoto, Japan). Sample preparation was performed using the potassium bromide (KBr) pellet method, where approximately 2 mg of each sample was uniformly mixed with approximately 200 mg KBr (FTIR-grade, purity ≥99%, Merck, Darmstadt, Germany). The mixture was pressed under vacuum to form a transparent pellet. Spectral data were collected at a resolution of 4 cm^{-1} with 32 scans per sample to ensure reproducibility and minimize noise. The instrument was calibrated

before each measurement, and background correction was applied to eliminate atmospheric interference. FTIR spectra were processed using IRsolution software (Shimadzu Corporation, Kyoto, Japan) without employing bucketing or data binning techniques. Preprocessing steps included baseline correction and normalization to enhance spectral clarity. The supplementary data, including raw FTIR spectral files, have been submitted to the journal to allow validation and authentication of the results. Each sample was measured once, and the data presented are from a single representative measurement.

Multivariate data analysis with chemometric techniques

The obtained spectra were analyzed using OriginLab and *The Unscrambler X 10.4 (Camo®)* software, employing chemometric techniques based on principal component analysis (PCA). Absorbance data within the mid-infrared region (4000–400 cm^{-1}) were selected and used as input for PCA. Before analysis, the data were preprocessed by normalization and mean-centering to minimize systematic errors and enhance interpretability. Sample measurements were conducted once without replication. Internal validation was carried out using the leave-one-out cross-validation (LOOCV) method to evaluate model stability and robustness. PCA was applied primarily for exploratory analysis and dimensionality reduction, rather than predictive modeling, to minimize the risk of overfitting. Limitations due to the small sample size were acknowledged, and suggestions for future studies involving larger datasets were provided.

The obtained Fourier Transform Infrared (FTIR) absorbance data at selected mid-infrared wavenumbers (4000–400 cm^{-1}) were used for multivariate data analysis employing PCA. This chemometric approach follows similar methodologies reported in previous studies using FTIR-PCA-PLS for oil authentication.[16] UHPLC-HRMS metabolomics for extract classification,[17] and FTIR-based essential oil profiling with PCA-HCA.[18] These references validate our use of PCA with internal leave-one-out cross-validation to assess model robustness.

Results and Discussion

FTIR spectra data

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify functional groups present in the extracts of *Citrus jambhiri* Lush. across eight samples, scanned within the mid-infrared region (4000–400 cm^{-1}). This analytical method offers rapid, non-destructive identification of chemical structures and has been widely used in authentication and phytochemical profiling of plant materials.[16,19,20] The resulting spectra (Figure 2) and corresponding absorbance peaks (Table 1) revealed common vibrations corresponding to O–H, C–H, C=O, C=C, and C–O bonds, supporting prior findings in similar matrices.[21,22]

Table 1: FTIR spectral data of all samples

Wave Number (cm^{-1})	Functional Groups	Sample								Ref
		P1	P2	A1	A2	K1	K2	D1	D2	
~3400-3490	O–H Stretch	3493	3462	3469	3441	3464	3414	3446	3406	[21–23,43,44]
~2950-2920	C–H Stretch (aliphatic)	2958	2956	2924	2929	2924	2929	2922	2926	
~1735-1710	C=O Stretch (carbonyl)	1732	1732	1743	1728	1735	1710	1735	1708	
~1220-1070	C–O Stretch (alcohol)	1141 1219	1126 1217	1163 1271	1072 1220	1126 1280	1072 1209	1120 1232	1070 1271	
~1600	C=C Stretch (aromatic)	1641	1639	1614	1631	1614	1614	1616	1614	
~3070-3000	C–H sp^2 Stretch (aromatic)	-	-	3007	-	3072	-	-	-	
~1450-1350	CH2 Wagging (aliphatic)	-	-	-	-	1458	1458	1454	1454	
~1380-1370	CH_3 Bending (Alkane)	1392	1400	1377	1400	1384	1398	1377	1398	

Note: P1 (ethyl acetate extract of *Citrus jambhiri* Lush juice); P2 (ethanol extract of *Citrus jambhiri* Lush juice); D1 (n-hexane extract of *Citrus jambhiri* Lush leaves); D2 (ethanol extract of *Citrus jambhiri* Lush leaves); A1 (n-hexane extract of *Citrus jambhiri* Lush fruit pulp); A2 (ethanol extract of *Citrus jambhiri* Lush fruit pulp); K1 (n-hexane extract of *Citrus jambhiri* Lush fruit peel); K2 (ethanol extract of *Citrus jambhiri* Lush fruit peel). The symbol "-" indicates the absence of absorption bands at the corresponding wavenumbers.

O–H stretching bands appeared consistently across all samples, with variations in wavenumber ranging from 3406 to 3493 cm^{-1}. Sample P1 showed the most intense peak at 3493 cm^{-1}, while D2 had the weakest at 3406 cm^{-1}. These differences suggest variation in hydroxyl-rich compounds such as polyphenols or saponins, a common feature in bioactive plant extracts.[21,23] Aliphatic C–H stretching, observed between 2920–2960 cm^{-1}, was prominent in samples P1 (2958 cm^{-1}) and P2 (2956 cm^{-1}), corresponding to CH_2 and CH_3 symmetric/asymmetric stretching. These peaks are typically linked to saturated hydrocarbon chains, such as those found in terpenoids or fatty acid derivatives.[22,24] Carbonyl (C=O) stretching bands were observed in the region of 1708–1743 cm^{-1}. Sample A1 exhibited the highest carbonyl peak at 1743 cm^{-1}, indicative of conjugated esters or strained cyclic compounds, while D2 had a lower shift (1708 cm^{-1}), suggesting less conjugated environments. Samples P1 and P2 showed consistent peaks at 1732 cm^{-1}, which strongly indicates the presence of esters or carboxylic acids.[21,24]

C=C aromatic stretching occurred between 1600–1641 cm^{-1}. P1 showed the highest shift at 1641 cm^{-1}, followed by P2 (1639 cm^{-1}) and

D1–D2 (1614–1616 cm^{-1}), indicating the presence of conjugated alkenes or aromatic ring systems.[23,25] Interestingly, A1 was the only sample exhibiting C–H sp^2 stretching at 3007 cm^{-1}, a feature associated with unsubstituted or mono-substituted aromatic structures, reinforcing its distinct profile.[25]

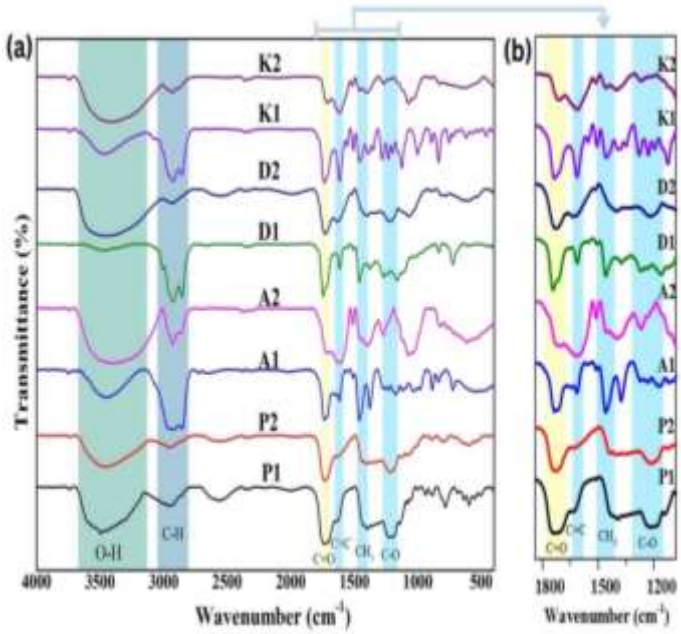

Figure 2: (a) FTIR spectrum pattern: P1 (ethyl acetate extract of *Citrus jambhiri* Lush juice); P2 (ethanol extract of *Citrus jambhiri* Lush juice); D1 (n-hexane extract of *Citrus jambhiri* Lush leaves); D2 (ethanol extract of *Citrus jambhiri* Lush leaves); A1 (n-hexane extract of *Citrus jambhiri* Lush fruit pulp); A2 (ethanol extract of *Citrus jambhiri* Lush fruit pulp); K1 (n-hexane extract of *Citrus jambhiri* Lush fruit peel); K2 (ethanol extract of *Citrus jambhiri* Lush fruit peel); **(b)** FTIR with wavenumber range 1800 cm^{-1} – 1050 cm^{-1}.

The C–O stretching region (1070–1271 cm^{-1}), related to alcohols, ethers, or ester linkages, showed variations among samples. A1 showed a strong absorption at 1271 cm^{-1}, whereas A2 and D1 had peaks at 1163 and 1232 cm^{-1}, respectively. These peaks support the presence of glycosidic linkages or ester functionalities, often encountered in flavonoid and phenolic glycosides.[22,26]

Bending vibrations such as CH_2 wagging (~1450–1350 cm^{-1}) and CH_3 bending (~1377–1400 cm^{-1}) were identified in several samples. CH_2 wagging was observed in A2, K1, and D2 at around 1454–1458 cm^{-1}, indicating methylene groups in long-chain aliphatic structures, while CH_3 bending was consistently observed, reflecting the presence of terminal methyl groups.[9,26]

Comparative analysis revealed that P1, P2, and A2 share similar chemical fingerprints, notably with intense peaks at ~1732 cm^{-1} (C=O), 3400–3490 cm^{-1} (O–H), and 2920–2950 cm^{-1} (C–H), supporting the presence of esters, hydroxylated aromatics, and potentially terpenoid or saponin compounds.[21,27] Meanwhile, K2, D1, and D2 displayed consistent peaks at ~3410, ~2925, ~1710, and ~1615 cm^{-1}, which align with polyphenolic or flavonoid structures previously identified using FTIR-chemometric combinations.[16,25]

Sample K1 demonstrated a rich and complete absorbance profile across all key regions, suggesting a chemically complex mixture containing terpenes, esters, phenolics, and possibly proteins. This is consistent with reports that have showed broad functionality in highly diverse extracts.[9] In contrast, sample A1 exhibited a unique peak at 3007 cm^{-1} for C–H sp^2 stretching, reinforcing its distinction due to aromatic content not observed in other extracts.[25]

Overall, the differences in FTIR absorbance regions, particularly those associated with O–H, C=O, and C=C bonds, reflect the influence of extraction solvent polarity on phytochemical composition. This supports prior findings that stated FTIR spectral signatures are strongly determined by solvent properties and extraction techniques, particularly when combined with chemometric tools for discrimination and authentication.[16,23]

Chemometric analysis outcome

This investigation employed chemometric analysis to examine the Fourier Transform Infrared (FTIR) spectral data of the *Citrus jambhiri* Lush plant diverse components. Chemometrics is a scientific discipline that utilizes mathematical and statistical methodologies to derive significant insights from chemical data. The integrated application of FTIR and chemometrics in this study provides a robust and efficacious strategy for the chemical characterization of *Citrus jambhiri* Lush, offering valuable insights for future research and the phytochemical products development.

Principal component analysis (PCA) one of the chemometric techniques employed in this study, was used to identify patterns and differences in spectral data as well as visualize the relationships between the samples being analyzed.[28] The use of PCA aims to reduce data complexity by transforming multidimensional spectral data into a few principal components that retain most of the variation in the data.[29] This approach facilitated the identification of major variations in chemical composition of the different parts of *Citrus jambhiri* Lush, enabling clearer visualization of differences influenced by factors such as the solvents used.

In addition to PCA, a dendrogram was used to perform hierarchical clustering analysis. The dendrogram served to classify samples based on their chemical similarities and differences as reflected in their FTIR spectra. This technique enabled a clearer interpretation of the relationships between samples by grouping those with similar chemical profiles and distinguishing those with significant differences. The dendrogram thus complemented PCA by revealing hierarchical relationships and patterns within the data that might not be evident through PCA alone. Chemometric analysis, particularly PCA and dendrograms, has proven effective in various studies for clustering and identifying chemical compounds in plants, as well as authenticating natural materials and their derivatives.[30]

Cluster interpretation

Cluster analysis based on the FTIR spectral data grouped the eight *Citrus jambhiri* Lush. extracts into three distinct clusters (Figure 3). These clusters reflect the influence of both the solvent polarity and the specific plant part used in the extraction process. Cluster 1 includes samples P1 and P2, which are juice extracts obtained using ethyl acetate and ethanol, respectively. These two samples formed a tight cluster with minimal linkage distance, indicating a high degree of similarity in their chemical profiles. This similarity is attributed to the solubility characteristics of the dominant compounds in citrus juice, such as flavonoid glycosides, organic acids, and sugars, which are soluble in both polar (ethanol) and semi-polar (ethyl acetate) solvents. Consequently, the use of different but closely related solvents does not lead to substantial differences in the FTIR profiles of the juice extracts. Similar findings were reported in previous studies. Rodrigues *et al*. (2021)[31] showed comparable phenolic profiles from orange juice extracted with polar solvents.[31] Ledesma- Ledesma -Escobar *et al.* demonstrated that varying ethanol-water ratios did not significantly alter the flavonoid composition in *Citrus limon* juice.[32] Likewise, a previous study observed minimal FTIR spectral differences between ethanol and ethyl acetate extracts of *Galium verum*, reinforcing that solvents with partially overlapping polarities tend to yield similar profiles.[33]

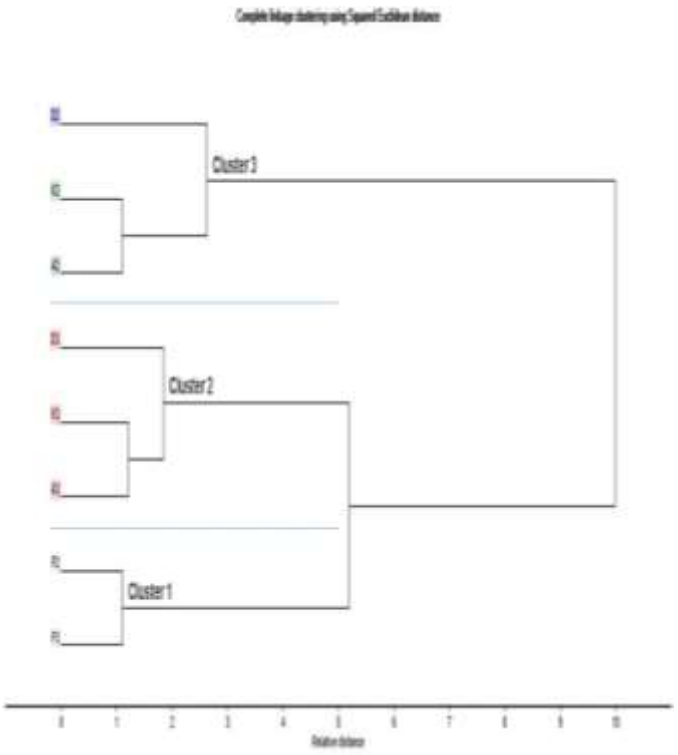

Figure 3: Dendrogram for classification of *Citrus jambhiri* Lush samples based on FTIR spectra using Cluster Analysis. The clustering was performed using the Squared Euclidean Distance and Complete Linkage method. Three main clusters were identified (dashed horizontal lines) at a relative distance of approximately 5: **Cluster 1**: P1 and P2 – juice extracts using ethyl acetate (P1) and ethanol (P2), **Cluster 2**: D1, K1, A1 – *n*-hexane extracts of leaves (D1), fruit peel (K1), and fruit pulp (A1), **Cluster 3**: D2, K2, A2 – ethanol extracts of leaves (D2), fruit peel (K2), and fruit pulp (A2).

Cluster 2, which comprises samples D1, K1, and A1, represents extracts obtained using n-hexane from different plant parts; leaves, peels, and pulp. The integration of these samples into one cluster with relatively short linkage distances highlights the consistency of n-hexane in extracting specific classes of compounds, particularly non-polar constituents. These typically include terpenoids, carotenoids, and volatile oils, which are widely distributed across citrus plant matrices. The effectiveness of n-hexane in extracting such compounds has been demonstrated in previous studies. Boukroufa *et al*. (2017) reported that n-hexane and d-limonene produced similar carotenoid-rich profiles from orange peels.[34] Similarly, Salvo et al. (2016) confirmed that n-hexane efficiently extracts essential oils and hydrophobic volatile compounds from citrus peels.[35] More recently, the role of n-hexane in extracting amide- and hydrocarbon-rich fractions in keratin-based matrices has been emphasized, supporting the consistent chemical profiles observed in Cluster 2.[24]

Cluster 3 includes samples D2, K2, and A2, all extracted with ethanol from different anatomical parts (leaves, peel, pulp). Despite their different origins, these samples exhibit highly similar chemical profiles, suggesting that ethanol selectively extracts a comparable range of polar compounds across tissues. Ethanol is known for its broad solvating capacity for polar phytochemicals, including flavonoids, phenolic acids, and ascorbic acid. This observation aligns with a study that reported that ethanol was more effective than methanol or acetone in extracting key flavonoids from *Citrus unshiu* pomace.[36] Similarly, another study demonstrated that ethanol yielded high phenolic content from citrus peels under enzyme-assisted conditions.[37] Supporting this, a chemometric profiling study revealed that methanol and ethanol extracts of citrus leaves produced overlapping FTIR signatures dominated by polar constituents.[25]

Overall, the FTIR-based clustering showed that solvent polarity was a more dominant factor than plant part in determining chemical similarity. Juice extracts (P1 and P2) formed a distinct cluster due to the unique metabolite composition of the juice. Meanwhile, different plant parts extracted with the same solvent (Cluster 2: hexane; Cluster 3: ethanol) consistently clustered together, underscoring the solvent's role in dictating extract composition. This supports the conclusion that solvent type significantly influences phytochemical extraction outcomes, and careful solvent selection is essential in targeted phytochemical applications.[38]

Sample interpretation on PCA scores plot

Figure 4 illustrates a plot of principal component analysis (PCA) scores, indicating the sample distribution by principal components (PC-1 and PC-2). PCA reduces the dimensionality of the data and extracts essential information while retaining significant variation in the data.[28] PC-1 (58%) is the first principal component explaining 58% of the total variation in the data. This is the horizontal axis on the plot. Simultaneously, PC-2 (23%) is the second principal component, explaining 23% of the total variation in the data. This is the vertical axis on the plot. These two principal components explain 81% of the total variation, suggesting that these two dimensions represent most of the data's vital information. This cumulative variance exceeds the generally accepted threshold (70–90%) for effective data representation in PCA.[39] The PCA results in Figure 4 show that P1 and P2 are located in the same quadrant, near the center axis of PC-1, indicating that the citrus juice extracts share similar chemical profiles. It aligns with the cluster analysis results (Figure 3), where P1 and P2 are also grouped. D1 and K1 are in the upper right quadrant, indicating similar chemical characteristics according to PC-1 and PC-2.

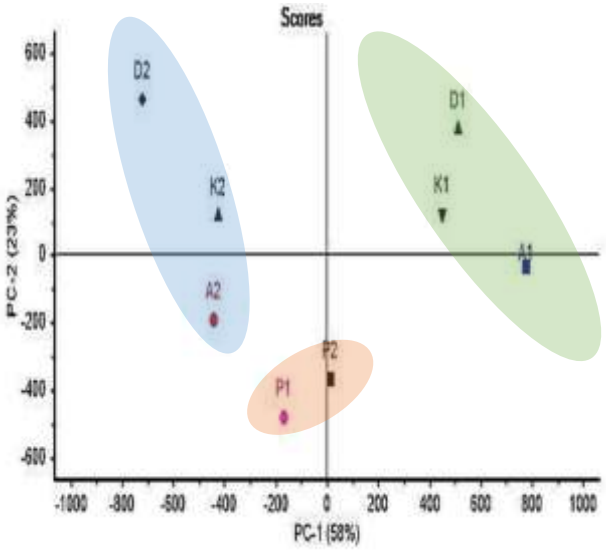

Figure 4: The score plot for classification of samples using PCA: P1 (ethyl acetate extract of *Citrus jambhiri* Lush juice); P2 (ethanol extract of *Citrus jambhiri* Lush juice); D1 (n-hexane extract of *Citrus jambhiri* Lush leaves); D2 (ethanol extract of *Citrus jambhiri* Lush leaves); A1 (n-hexane extract of *Citrus jambhiri* Lush fruit pulp); A2 (ethanol extract of *Citrus jambhiri* Lush fruit pulp); K1 (n-hexane extract of *Citrus jambhiri* Lush fruit peel); K2 (ethanol extract of *Citrus jambhiri* Lush fruit peel)

This is consistent with the cluster analysis (Figure 3), where D1 and K1 are grouped, suggesting that n-hexane solvent produces a similar extraction profile from the leaves and peel of *Citrus jambhiri* Lush. The similarity between D1 (n-hexane extract of leaves) and K1 (n-hexane extract of peel) suggests that non-polar solvents such as n-hexane tend to extract comparable classes of non-polar compounds (e.g., terpenoids, fatty acids, and waxes) from different plant tissues that share similar

epidermal or cuticular structures. Previous studies have reported that plant leaves and fruit peels, particularly in Citrus species, possess similar lipid-based metabolites and cuticular compounds when extracted using non-polar solvents.[40] This chemical similarity may explain their clustering in both PCA and dendrogram analysis. A1 is located near D1 and K1, indicating chemical similarity with the n-hexane extracts of *Citrus jambhiri* Lush leaves and peels. This is also consistent with the cluster analysis results. D2 and K2 are positioned in the upper left quadrant, slightly separated from A2, indicating that although they were extracted with ethanol, the leaf and peel extracts possess distinct chemical profiles compared to the fruit pulp extract. While D2 and K2 cluster together, differences in their PCA positioning highlight subtle compositional variations. A2, situated in the lower left quadrant and distant from D2 and K2, indicates significant differences in the chemical composition despite being grouped with them in the cluster analysis. This suggests that the anatomical source of the extract strongly influences its chemical profile, even when using the same solvent.

The PCA results (Figure 4) and the cluster analysis (Figure 3) demonstrate consistency. Samples grouped in the cluster analysis (such as P1-P2 and D1-K1-A1) also exhibited proximity in the PCA scores plot. One notable difference in the PCA was the separation within the ethanol group, where D2, K2, and A2 were more distinctly distributed, despite being grouped together in the cluster analysis. This indicates that PCA can reveal more subtle differences in the chemical profiles of the extracts, which might not be fully captured by cluster analysis.[3]

PCA provides a more precise visualization of the differences and similarities between samples in a two-dimensional space, broadly consistent with the cluster analysis results. The P1-P2 and D1-K1-A1 clusters identified by the cluster analysis were confirmed by PCA, indicating strong chemical similarity in these extracts. The positioning of D2, K2, and A2 in PCA illustrates that, despite their inclusion in the same broader category in the cluster analysis, distinct differences among them are more clearly revealed in PCA. Consequently, both PCA and cluster analysis provide valuable insight into the interrelations between samples, with PCA possessing the capacity to underscore more nuanced variations in the chemical profiles.[41,42]

Conclusion

This study demonstrated that Fourier Transform Infrared (FTIR) spectroscopy combined with chemometric techniques *vis-à-vis* principal component analysis (PCA) and hierarchical clustering effectively characterizes the chemical composition of various *Citrus jambhiri* Lush. extracts. The dominant functional groups identified were O–H, C–H, C=O, and C=C, indicating the presence of bioactive compounds such as flavonoids, terpenoids, and aromatic esters. Cluster analysis revealed that solvent type had a greater influence on extract composition than plant part. Notably, n-hexane extracts (from leaves, peel, and pulp) clustered together due to their shared non-polar constituents, while ethanol extracts showed more intra-group variation depending on tissue type. Juice extracts formed a distinct cluster, suggesting the presence of unique chemical constituents. These findings support the strategic application of solvent selection to isolate specific phytochemical classes from *Citrus jambhiri* Lush. For future studies, a more comprehensive metabolomic approach such as coupling FTIR with Liquid Chromatography - Mass Spectrometry (LC-MS) or Nuclear Magnetic Resonance (NMR) could be applied to validate compound identities and quantify phytochemicals across extracts. In addition, bioactivity-guided fractionation could be explored to correlate specific spectral patterns with biological functions, supporting the development of functional products or standardized herbal formulations.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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