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A Sustainable ATR-FTIR Spectroscopy Approach Coupled with AI-Driven Chemometrics for Rapid Quality Assessment of Kanchanar Guggulu Formulations

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Research Article

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Abstract

Background

Kanchanar Guggulu (KG) is a traditional Ayurvedic polyherbal formulation commonly used for the management of glandular and metabolic disorders such as benign prostatic hyperplasia, polycystic ovary syndrome, hypothyroidism, and fibroadenomas. However, the quality evaluation of commercially available KG products remains challenging because of variability in raw materials, manufacturing procedures, and storage conditions. Therefore, rapid and environmentally sustainable analytical approaches are required to ensure product quality and consistency.

Methods

This study developed a rapid analytical strategy using attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy to characterize the chemical fingerprint profiles of KG formulations. Thirteen samples, including one authenticated in-house reference sample, were analyzed. Spectral data were collected within the range of 4000–400 cm^{-1} and processed using chemometric tools, particularly principal component analysis (PCA), to evaluate sample classification and spectral variability. The environmental sustainability of the method was assessed using Complex Green Analytical Procedure Index (Complex GAPI), Analytical GREEnness (AGREE), the Blue Applicability Grade Index (BAGI), and a RGB12 model.

Results

ATR-FTIR fingerprinting successfully captured characteristic spectral patterns of KG formulations, while PCA enabled clear differentiation among commercial samples. Sustainability evaluation indicated high performance across all assessment tools, demonstrating minimal environmental impact, reduced reagent use, and strong analytical applicability.

Conclusions

The integration of ATR-FTIR spectroscopy with Artificial Intelligence-driven chemometric analysis provides a rapid, cost-effective, and environmentally responsible approach for routine quality assessment of Ayurvedic formulations, highlighting the potential of data-driven analytical strategies in advancing research within traditional and complementary medicine.

Introduction

Traditional herbal medicine has long played a vital role in healthcare systems across the world. Among these, Ayurveda represents one of the oldest holistic medical traditions, emphasizing the use of plant-

based formulations for disease prevention and treatment. Herbal preparations derived from medicinal plants are commonly administered in various dosage forms such as powders, tablets, capsules, and decoctions. These phytomedicines are widely utilized due to their accessibility, affordability, and cultural acceptance in several countries including India, China, Korea, and Japan [1, 2]. In recent decades, the growing global interest in Traditional, Complementary, and Integrative Medicine has highlighted the importance of scientifically validating and standardizing traditional herbal formulations to ensure their safety, quality, and therapeutic effectiveness [3].

One of the classical polyherbal formulations described in Ayurvedic literature is Kanchanar Guggulu, which is traditionally prescribed for the management of glandular swellings and metabolic disorders. The formulation has been widely used in the treatment of conditions such as Benign Prostatic Hyperplasia, Polycystic Ovary Syndrome, Hypothyroidism, and fibroadenomas of the breast [4, 5]. In Ayurvedic therapeutics, these conditions are often associated with abnormal tissue growth or glandular enlargement, and Kanchanar Guggulu is believed to exert beneficial effects by promoting metabolic balance, supporting detoxification pathways, and reducing pathological tissue accumulation. Due to these therapeutic properties, the formulation continues to be widely used in modern Ayurvedic clinical practice [6–10]

As a testament to its versatility, Kanchanar Guggulu demonstrates promising cytotoxic properties and proves beneficial in mitigating symptoms related to these conditions without the adverse effects typically observed with invasive procedures and contemporary medicines[11]. Additionally, treatment with Kanchnar Guggulu has demonstrated significant relief in symptoms associated with prostate enlargement, as indicated by International Prostate Symptom Score (IPSS) scores [12]

Despite its extensive medicinal use, ensuring the quality and authenticity of herbal formulations such as Kanchanar Guggulu remains a significant challenge. Variability in raw materials, geographical origin of medicinal plants, manufacturing processes, and storage conditions can influence the chemical composition of herbal products, potentially affecting their safety and efficacy [13]. Therefore, reliable analytical techniques are essential for the standardization and quality control of these complex herbal matrices. Spectroscopic techniques, particularly Attenuated Total Reflection Fourier Transform Infrared Spectroscopy, have emerged as rapid and non-destructive tools for the characterization of herbal formulations. ATR-FTIR spectroscopy provides molecular fingerprint information of samples with minimal sample preparation, making it highly suitable for routine analysis and authentication of traditional medicines [14].

To further enhance the interpretation of spectroscopic data, advanced multivariate analytical approaches such as Chemometrics are increasingly being applied. Techniques like Principal Component Analysis (PCA) enable the extraction of meaningful patterns from complex spectral datasets and allow the differentiation of authentic and commercial samples. Importantly, these chemometric methods are increasingly integrated with Artificial Intelligence, which can enhance pattern recognition, classification, and predictive modelling in herbal medicine research. The integration of artificial intelligence with

spectroscopic and chemometric tools is gaining attention within TCIM research as it enables more accurate quality assessment, authentication, and standardization of traditional herbal products [15].

Herbal medicines have experienced a significant rise in global use in recent years [16]. However, phytochemical investigations of herbal materials available in the Indian market have indicated that nearly 60% of the tested samples show some form of adulteration or substitution. Such findings highlight the urgent need for reliable quality control and standardization of Ayurvedic raw materials and formulations [17–19]. Despite the wide range of therapeutic benefits associated with Kanchanar Guggul, herbal products of this type are also susceptible to adulteration during commercial distribution [20].

Kanchanar Guggul, a classical Ayurvedic formulation, contains multiple botanical ingredients that contribute to its therapeutic activity. Variations in the chemical composition of these plant components may occur due to differences in plant parts, stages of development, anatomical characteristics, and their biological roles within the plant life cycle. These variations can influence the concentration and distribution of bioactive phytochemicals in the formulation. Because of such chemical diversity, relying solely on macroscopic examination or organoleptic evaluation can be insufficient, as these approaches depend largely on the observer's sensory perception and may not reliably detect subtle compositional differences [21, 22]. Therefore, an eco-friendly and rapid chemometrics-assisted ATR-FTIR method was developed in this study to differentiate Kanchanar Guggul samples based on their chemical profiles. This analytical strategy can further assist in detecting compositional variations among different batches and contribute to the quality evaluation of the formulation.

Conventional analytical techniques often face challenges in aligning with the principles of sustainable chemistry because they frequently involve hazardous reagents, complex procedures, and costly instrumentation. These factors can negatively impact human health, environmental safety, and economic feasibility. Consequently, the integration of the principles of Green Analytical Chemistry (GAC) and Blue Analytical Chemistry (BAC) has become increasingly important in modern analytical practices [23]. Many existing food and herbal analytical approaches still contradict these sustainability principles by presenting considerable environmental, health, and financial burdens. In addition, no comprehensive analytical method has yet been reported for evaluating the greenness, blueness, and whiteness characteristics of Kanchanar Guggul.

Therefore, in the present study, ten samples of Kanchanar Guggul were analyzed using Fourier Transform Infrared Spectroscopy with Attenuated Total Reflection (ATR-FTIR). Chemometric analysis was applied to the obtained spectral data, and an unsupervised classification technique, Principal Component Analysis (PCA), was employed to construct the analytical model. In addition to analytical reliability, modern research also emphasizes environmental sustainability in analytical chemistry. Concepts such as analytical greenness, blueness, and whiteness have been introduced to evaluate the ecological impact, practical efficiency, and overall sustainability of analytical methods. These metrics assess parameters such as reagent consumption, waste generation, energy usage, and analytical performance to promote environmentally responsible laboratory practices [24].

In this context, the present study aims to develop and evaluate a rapid and environmentally sustainable analytical approach based on ATR-FTIR spectroscopy coupled with chemometric analysis for the characterization of Kanchanar Guggulu samples. By integrating spectroscopic fingerprinting, chemometric interpretation, and sustainability assessment metrics, the proposed method offers a simple, rapid, precise, and eco-friendly strategy for the routine quality control and differentiation of commercial formulations of this important Ayurvedic medicine.

Materials and Methods

Materials and Sample Collection

A total of thirteen commercial samples of Kanchanar Guggulu (KG) were collected from different Ayurvedic pharmacies, herbal medicine retailers, and online distributors across Gujarat, India. In addition to the market samples, one authenticated in-house KG formulation prepared according to classical Ayurvedic guidelines was included as a reference standard for comparative analysis. All samples were coded and stored in airtight containers at ambient laboratory conditions prior to analysis. Each sample was assigned a unique colour code for graphical identification in spectral correlation and chemometric visualization plots.

Table 1 Sources of Kanchanar Guggulu samples collected for the study

Sr. No.	Source of Sample
1	Atharva ayurveda
2	Patanjali Ayurveda
3	Baidyanath Asli Ayurveda
4	Authenticated in-house sample
5	Zandu Pharmaceutical
6	Dabur Ayurvedic
7	Charak Pharma
8	Zandu Pharmacy
9	Sandu Pharmaceuticals
10	Planet Ayurveda
11	Dhootapapeshwar
12	Patanjali Ayurveda
13	Satnam Ayurvedic stores
14	Narayan Ayurvedic stores

Authentication of the Reference Sample

Preparation of In-House Kanchanar Guggulu

The reference formulation of Kanchanar Guggulu tablets were prepared following traditional Ayurvedic methods described in recognized pharmacopeial and classical texts. It was prepared by accurately weighing and homogenizing Kanchnar (*Bauhinia variegata*, 80 g), Haritaki (*Terminalia chebula*, 16 g), Vibhitaki (*Terminalia bellirica*, 16 g), Amlaki (*Phyllanthus emblica*, 16 g), Pippali (*Piper longum*, 8 g), Marica (*Piper nigrum*, 8 g), Sunthi (*Zingiber officinale*, 8 g), Varuna (*Crataeva nurvala*, 8 g), Twak (*Cinnamomum zeylanicum*, 2 g), Shukshmaila (*Elettaria cardamomum*, 2 g), Tejapatra (*Cinnamomum tamala*, 2 g), and Guggulu (*Commiphora mukul*, 166 g), then blended with a paste of Carboxymethyl Cellulose Sodium (1% w/w), Gum Acacia (5% w/w), Liquid Glucose (5% w/w), and water, with 0.5% Aerosil added as an anticaking agent, dried, and finally compressed into tablets for storage at room temperature. These established procedures were adopted to ensure that the prepared formulation closely reflected the authentic composition and processing techniques used in Ayurvedic practice within the broader domain of Traditional, Complementary, and Integrative Medicine. Preparing a well-characterized reference sample is an important step in analytical studies because it serves as a standard for comparing the quality and consistency of commercially available products. The formulation was composed of medicinal plant ingredients traditionally associated with Kanchanar Guggulu along with other herbal constituents that are typically incorporated in the classical preparation. These botanical ingredients are known in Ayurvedic literature for their potential roles in supporting metabolic balance, reducing glandular swelling, and managing conditions involving abnormal tissue growth.

All raw plant materials were obtained from certified and reputable herbal suppliers to ensure traceability and quality. After procurement, each botanical ingredient underwent taxonomic authentication by a qualified botanist at a recognized botanical research institute. This authentication step was carried out through careful examination of morphological characteristics and comparison with authenticated herbarium specimens. Such verification is essential in herbal medicine research because misidentification or substitution of plant materials can significantly influence the therapeutic efficacy and chemical profile of the final formulation.

Following authentication, the plant materials were thoroughly cleaned to remove extraneous matter such as soil particles, dust, and other foreign contaminants that might interfere with subsequent analytical evaluation. The cleaned materials were then dried under controlled shade conditions at ambient temperature. Shade drying was intentionally selected because exposure to excessive heat or direct sunlight can lead to degradation of sensitive phytochemicals, including phenolic compounds, volatile constituents, and other bioactive metabolites present in medicinal plants.

Once the plant materials were completely dried, they were pulverized using a laboratory-grade mechanical grinder to obtain a coarse but uniform powder. Powdering enhances the surface area of the plant material, which facilitates uniform blending and improves the homogeneity of the formulation. The powdered ingredients were subsequently weighed according to the traditional proportions specified in

Ayurvedic pharmacopeial references. Careful weighing ensured that each component was incorporated in the correct ratio, thereby maintaining the integrity of the classical formulation.

After weighing, the powdered herbal ingredients were thoroughly blended to produce a uniform mixture. Homogenization was performed using mechanical mixing to ensure even distribution of all constituents throughout the formulation. Achieving a homogeneous blend is essential for ensuring that each unit dose of the final product contains consistent proportions of the herbal components. The prepared mixture was then processed further for tablet preparation according to standard Ayurvedic pharmaceutical practices, yielding the authenticated reference sample used in the subsequent analytical and spectroscopic evaluation.

Physicochemical Evaluation

The physicochemical characteristics of the reference formulation of Kanchanar Guggulu were systematically examined to confirm its authenticity and overall quality prior to spectroscopic analysis. Determination of these parameters is an essential step in the standardization of traditional herbal formulations within the framework of Traditional, Complementary, and Integrative Medicine. Such measurements provide important information regarding the purity, composition, and physical properties of the formulation and serve as baseline indicators for comparison with commercial samples.

In-house prepared Kanchanar Guggul tablets were prepared and evaluated for key pharmaceutical quality control parameters to ensure their uniformity, mechanical strength, and proper performance including organoleptic parameters. The weight variation test was conducted to confirm uniform distribution of the formulation in each tablet and to ensure dose consistency. Friability testing was performed to assess the mechanical resistance of the tablets against abrasion and breakage during handling, packaging, and transportation. In addition, the disintegration time test was carried out to determine the time required for the tablets to break down into smaller particles under simulated physiological conditions, which is essential for proper drug release and therapeutic effectiveness. These parameters collectively help in confirming the quality and suitability of the prepared tablets for further evaluation.

In the present study, Physicochemical parameters like total ash, acid insoluble ash and loss on drying at 105◦C, alcohol, and water-soluble extractive values were carried out as per the API/WHO guidelines. All experimental measurements were performed in triplicate to ensure accuracy and reproducibility, and the results were expressed as mean values accompanied by the corresponding standard deviation (mean ± SD).

Together, these physicochemical evaluations provide a comprehensive assessment of the basic quality attributes of the reference Kanchanar Guggulu formulation. Establishing these parameters ensures that the reference material used for subsequent spectroscopic and chemometric analysis accurately represents a standardized formulation, thereby strengthening the reliability of analytical comparisons and quality control studies.

ATR-FTIR Spectroscopic Analysis

Chemical fingerprinting of all samples of Kanchanar Guggulu was carried out using Attenuated Total Reflection Fourier Transform Infrared Spectroscopy. This analytical technique was selected because it allows rapid characterization of complex herbal formulations by generating a comprehensive spectral profile that reflects the overall chemical composition of the sample. In studies involving polyherbal medicines within Traditional, Complementary, and Integrative Medicine, spectroscopic fingerprinting has become an increasingly valuable approach for quality evaluation, as it enables the identification of characteristic functional groups without requiring extensive sample preparation.

Prior to spectral acquisition, each powdered formulation was carefully prepared to ensure analytical consistency. All samples were passed through a 60-mesh sieve in order to obtain a uniform particle size distribution. This step is important in ATR-FTIR analysis because variations in particle size can influence the interaction between the sample surface and the infrared beam, which may subsequently affect spectral intensity and reproducibility. Achieving a consistent particle size therefore helps ensure that the spectra obtained from different samples are comparable and reliable. After sieving, the powdered materials were transferred directly to the ATR sampling accessory for analysis. No solvent extraction or chemical treatment was performed, as direct analysis of the powdered samples allows preservation of the natural chemical fingerprint of the formulation. This approach also reduces analytical time and avoids the potential loss or modification of sensitive phytochemicals that may occur during extraction procedures.

Infrared spectra were recorded using a Fourier transform infrared spectrometer equipped with an ATR crystal accessory designed for solid sample analysis. During measurement, a small quantity of powdered sample was placed on the surface of the ATR crystal and gently pressed to ensure optimal contact between the sample and the crystal surface. The infrared beam then interacted with the sample through internal reflection within the crystal, producing a spectrum representing the vibrational frequencies of the functional groups present in the formulation. Spectral acquisition was carried out over the mid-infrared region ranging from 4000 to 400 cm^{-1}, which encompasses the most informative absorption bands associated with organic functional groups such as hydroxyl, carbonyl, aromatic, and aliphatic structures commonly found in plant-derived compounds. A spectral resolution of 4 cm^{-1} was employed to achieve a suitable balance between spectral clarity and data acquisition speed, while sixteen scans were recorded for each sample to improve the signal-to-noise ratio and enhance spectral reliability.

To evaluate the reproducibility of the analytical method, replicate measurements were initially performed using the authenticated in-house reference sample of Kanchanar Guggulu. Six independent spectra were recorded under identical experimental conditions. Comparison of these replicate spectra demonstrated highly consistent absorption patterns and identical functional group regions across all measurements, indicating excellent reproducibility and stability of the ATR-FTIR method for the analysis of the formulation. This confirmation step was important to ensure that any spectral differences observed

among commercial samples could be attributed to variations in composition rather than instrumental variability.

Following spectral acquisition, the collected data were subjected to preliminary preprocessing before multivariate statistical analysis. Baseline correction was applied to remove background fluctuations and improve the clarity of absorption peaks. Additional spectral preprocessing steps, including normalization and smoothing, when necessary, were performed using specialized spectral analysis software. These preprocessing procedures help enhance spectral comparability and reduce noise, thereby enabling more accurate interpretation of the chemical fingerprints during subsequent chemometric analysis. The processed spectra were then used as input data for advanced multivariate techniques, facilitating objective comparison and quality evaluation of different Kanchanar Guggulu samples [25, 26].

Chemometric Analysis and AI-Assisted Pattern Recognition

The spectral data generated from the analysis of Kanchanar Guggulu using Attenuated Total Reflection Fourier Transform Infrared Spectroscopy were further examined through multivariate statistical analysis to extract meaningful chemical information from the complex spectral patterns. Spectra obtained from polyherbal formulations often contain a large number of overlapping absorption bands corresponding to diverse phytochemical constituents. Interpreting such multidimensional data through simple visual comparison can be challenging and may not effectively reveal subtle differences among samples. Therefore, a chemometric approach was employed to systematically analyze the spectral dataset and identify patterns that could assist in the comparative quality assessment of different samples. Chemometrics integrates statistical and computational tools to analyze large analytical datasets, and its application is increasingly recognized as an important analytical strategy within Traditional, Complementary, and Integrative Medicine.

Principal Component Analysis

For the purpose of dimensionality reduction and pattern recognition, Principal Component Analysis (PCA) was applied to the ATR-FTIR spectral dataset using Minitab statistical software. PCA is widely used in spectroscopic studies because it converts a large number of correlated variables into a smaller set of new variables known as principal components. These components are mathematically derived combinations of the original spectral variables and are arranged in such a way that they capture the maximum possible variance present in the dataset. The first principal component (PC1) explains the greatest proportion of variability in the spectral data, while the second principal component (PC2) accounts for the next highest amount of variation. Subsequent components describe progressively smaller contributions of variance. By projecting complex spectral information onto a limited number of principal components, PCA enables the visualization of relationships among samples and simplifies interpretation of the dataset.

To better understand the structure of the data and identify patterns among the analyzed samples, several graphical visualization tools associated with PCA were employed. Scree plots were first

generated to evaluate the relative contribution of each principal component and to determine the number of components that meaningfully represent the variation within the dataset. These plots display the eigenvalues associated with each principal component and help identify the point at which additional components contribute only minimal information. Score plots were then constructed to visualize the distribution and clustering of samples based on their principal component scores. These plots provide a graphical representation of similarities or differences among the analyzed samples, allowing potential grouping patterns or outliers to be identified. In addition, loading plots were examined to determine which spectral variables or wavenumber regions contributed most strongly to the observed variability. These plots help reveal the specific functional group regions responsible for differences between samples and therefore provide insights into potential compositional variations within the formulation.

The combination of ATR-FTIR spectroscopy with chemometric analysis thus represents a powerful analytical strategy for evaluating complex herbal formulations. By transforming large spectral datasets into interpretable patterns, PCA enables objective comparison of multiple samples and facilitates the identification of subtle variations in their chemical profiles. This data-driven analytical workflow also reflects the increasing role of Artificial Intelligence in modern analytical science. Although PCA itself is an unsupervised statistical method, its integration with advanced computational tools forms part of an AI-assisted analytical framework that can support automated detection of compositional differences in herbal medicines. Such approaches are particularly valuable for improving quality control, authenticity verification, and standardization of traditional polyherbal formulations used in TCIM research and pharmaceutical development [27-30].

Results

Authentication of the Reference Kanchanar Guggulu

The physicochemical evaluation of the in-house prepared Kanchanar Guggulu demonstrated that the formulation adhered closely to established pharmacopeial standards for Ayurvedic medicines. These parameters are widely recognized as essential benchmarks for assessing the quality, purity, and authenticity of herbal preparations. Maintaining these parameters within recommended limits is crucial not only for ensuring the safety and efficacy of the formulation but also for preserving the integrity of its bioactive compounds.

All the parameters were well within the prescribed limits confirming the care taken in the collection and processing of raw materials. These results collectively suggest that the traditional preparation methods used for the in-house Kanchanar Guggulu formulation were appropriately executed, ensuring both the purity and reproducibility of the final product. Importantly, these findings validate the suitability of the in-house formulation as a reliable reference standard for subsequent analytical studies. By establishing that the physicochemical characteristics conform to pharmacopeial benchmarks, the reference formulation provides a robust baseline for comparative quality assessment against commercially available Kanchanar Guggulu products. This reliability is critical for the interpretation of spectroscopic

and chemometric analysis, as it allows deviations in commercial samples to be accurately attributed to differences in composition, processing, or adulteration, rather than methodological inconsistencies. In essence, the adherence of the in-house formulation to established quality standards underscores its value as a trusted reference material for both research and quality control within the framework of traditional, complementary, and integrative medicine (TCIM).

Table 2 Physicochemical properties of in house Kanchanar Guggulu samples

Sr.no	Parameter	Result
1	Organoleptic evaluation	Colour: brownish, Odor: characteristic smell
2	Weight variation	Within limits
3	Friability	0.42±0.03%
4	Disintegration time	14 min
5	Total ash	5.03±0.40%
6	Acid insoluble ash	1.90±0.02%
7	Alcohol soluble extractive	26.03±0.02%
8	Water soluble extractive	25.26±2.83%
9	Loss on drying	4.38±0.02

All values are uttered as mean ± SD (n =6).

ATR-FTIR Spectral Fingerprinting

The ATR-FTIR analysis of all Kanchanar Guggulu samples produced well-defined and reproducible spectral fingerprints, providing a detailed chemical profile of the formulations. The spectra revealed several prominent absorption bands, each corresponding to specific functional groups commonly found in plant-derived bioactive compounds. Broad absorption in the region of 3200–3600 cm^{-1} was observed, indicative of O–H stretching vibrations typically associated with hydroxyl groups. These groups are abundant in phenolic compounds, flavonoids, and other polyhydroxy constituents, which contribute significantly to the therapeutic properties of the formulation. Peaks in the range of 2800–3000 cm^{-1} were attributed to C–H stretching vibrations, reflecting the presence of aliphatic chains, terpenoids, and other hydrocarbon moieties commonly found in resinous and oily components of the polyherbal blend.

A distinct absorption band near 1700 cm^{-1} corresponded to C=O stretching vibrations, which are characteristic of carbonyl-containing compounds such as ketones, aldehydes, and carboxylic acids. These functional groups are frequently found in flavonoid glycosides, phenolic acids, and other

secondary metabolites that contribute to the pharmacological activity of the formulation. Furthermore, absorption in the region of 1000–1300 cm^{-1} was assigned to C–O stretching vibrations, which can be linked to alcohols, ethers, esters, and glycosidic linkages present in polysaccharides and other complex phytoconstituents. Jasco 4700 ATR for spectra acquisition, along with software tools including Minitab. The spectra were recorded over a range of 400–4000 cm^{-1}. Spectra were acquired with 16 co-added scans at 4 cm^{-1} spectral resolution.

Taken together, the observed ATR-FTIR peaks reflect the diverse phytochemical composition of Kanchanar Guggulu, including flavonoids, phenolic compounds, terpenoids, and resin constituents derived from multiple medicinal plant ingredients. These spectral features serve as a unique chemical fingerprint, enabling the identification and differentiation of authentic formulations from commercially available products. The comprehensive fingerprinting not only provides a non-destructive method for rapid quality assessment but also establishes a foundation for subsequent chemometric analyses, facilitating objective comparison of sample composition, consistency, and overall quality in alignment with modern analytical approaches in traditional, complementary, and integrative medicine (TCIM).

Chemometric Evaluation of Spectral Data

Principal Component Analysis

Principal Component Analysis (PCA) was applied to the ATR-FTIR spectral data specifically focusing on the fingerprint region, which encompasses the most informative absorption bands associated with the complex phytochemical constituents of Kanchanar Guggulu. A total of seventy-two spectral variables, representing key wavenumbers within this region, were included in the analysis to comprehensively capture the chemical variability among all tested samples. By transforming the high-dimensional spectral dataset into a smaller set of uncorrelated variables known as principal components, PCA allowed the reduction of data complexity while retaining the most relevant chemical information.

Examination of the resulting scree plot revealed that the fourteen principal components collectively accounted for the whole of the variance present in the spectral dataset of important 67 peaks. First two components summarized the main sources of variation (near to 40%) among the samples, effectively condensing the multidimensional spectral information into a lower-dimensional space that is easier to interpret and visualize. The dominance of the first two principal components also suggests that the chemical differences between samples are concentrated in specific spectral regions corresponding to key functional groups, while minor variations are captured by subsequent components.

The PCA score plot provided a clear visual representation of the chemical relationships among the commercial Kanchanar Guggulu samples relative to the authenticated in-house reference formulation. Most of the commercial samples were observed to cluster closely around the reference point, indicating that these products shared a chemical composition highly similar to the standard formulation. This clustering suggests consistency in the proportion and quality of the phytochemical constituents, reflecting adherence to traditional preparation methods and proper sourcing of plant materials in these

products. The close alignment of these samples with the reference also demonstrates the reliability of ATR-FTIR fingerprinting coupled with PCA as a robust analytical approach for assessing formulation authenticity in polyherbal Ayurvedic medicines. Samples (1, 13, and 14) with dissimilarity are represented with red colour symbols and samples (7, 10, and 12) with lesser similarity are represented with blue hollow circles.

Conversely, two of the commercial samples were positioned at a considerable distance from the main cluster in the score plot. This deviation implies potential variations in their chemical composition compared to the reference standard. Such differences could arise from multiple factors, including inconsistencies in raw material quality, variations in processing techniques, substitution or adulteration of ingredients, or deviations in the ratios of constituent herbs. These outlying samples highlight the importance of rigorous quality control in herbal formulations, as even minor alterations in composition can affect both the therapeutic efficacy and safety profile of the product. The ability of PCA to detect these variations underscores the utility of chemometric methods as an AI-assisted tool for rapid and objective evaluation of complex herbal medicines, supporting informed decision-making in quality assurance and standardization efforts within the framework of traditional, complementary, and integrative medicine (TCIM).

Overall, the PCA results demonstrate that the chemometric approach provides a robust framework for analyzing complex ATR-FTIR data of Kanchanar Guggulu formulations. By reducing the dimensionality of the dataset without significant loss of information, PCA facilitates the identification of patterns, clustering of similar samples, and detection of outliers. This dimensionality reduction is particularly valuable when working with polyherbal formulations in traditional, complementary, and integrative medicine (TCIM), where the inherent chemical complexity often makes direct spectral comparison challenging. In this way, PCA serves as a critical AI-assisted tool for extracting meaningful insights from spectral data, supporting objective quality assessment and standardization of herbal products.

The PCA results highlight the usefulness of ATR-FTIR coupled with chemometric tools as a **rapid quality screening method** for Ayurvedic formulations.

Artificial Intelligence–Assisted Spectral Data Interpretation

To further enhance the interpretation of complex spectral datasets obtained from Attenuated Total Reflection Fourier Transform Infrared Spectroscopy, the present study incorporates an artificial intelligence–assisted analytical framework based on Chemometrics. Spectroscopic techniques typically generate large multivariate datasets containing overlapping signals from numerous phytochemical constituents present in polyherbal formulations such as Kanchanar Guggulu. Traditional univariate approaches often fail to capture subtle variations in such complex matrices. Therefore, multivariate PCA - based pattern recognition techniques are increasingly being applied in herbal medicine research to enable efficient classification, authentication, and quality evaluation.

In the current investigation, Principal Component Analysis (PCA) was employed as an unsupervised machine learning algorithm to identify hidden structures and clustering patterns within the ATR-FTIR spectral dataset. PCA transforms high-dimensional spectral variables into a smaller set of orthogonal components while preserving the majority of data variance. This dimensionality reduction enables effective visualization of relationships among samples and facilitates detection of outliers or samples with altered chemical composition. By projecting the spectral fingerprints into principal component space, PCA allows automated recognition of similarities and differences among commercial formulations relative to the authenticated reference sample.

Sustainability Assessment of the Analytical Method

Greenness Evaluation

The environmental sustainability of the developed analytical workflow for Kanchanar Guggulu was systematically assessed using two widely recognized green chemistry metrics: the AGREE metric and the Complex Green Analytical Procedure Index (Complex GAPI). Both tools are grounded in the principles of Green Analytical Chemistry and provide quantitative and visual measures of how environmentally friendly an analytical method is. The ATR-FTIR-based method exhibited outstanding greenness due to several key factors. First, it required minimal reagent consumption, significantly reducing chemical waste generation. Second, no hazardous or toxic solvents were employed at any stage, eliminating potential risks to both laboratory personnel and the environment. Third, the method streamlined sample handling and preparation, avoiding labor-intensive extraction procedures that are common in traditional chromatographic techniques. Finally, the energy requirements were inherently low, as the ATR-FTIR measurements could be performed directly on powdered samples without heating, derivatization, or other energy-intensive steps. Collectively, these attributes confirm that the proposed method aligns with sustainable analytical practices and minimizes ecological impact, making it a highly eco-friendly approach for routine quality control of herbal formulations [31].

Blueness Evaluation

In addition to environmental considerations, the practical applicability of the method—often referred to as “blueness”—was evaluated using the Blue Applicability Grade Index (BAGI). This tool quantifies how fit-for-purpose an analytical method is in real-world laboratory scenarios, considering parameters such as analysis time, ease of automation, sample throughput, instrumentation complexity, and operator requirements. The ATR-FTIR method for Kanchanar Guggulu scored highly on the BAGI scale, reflecting its suitability for routine application in quality control laboratories. The rapid spectral acquisition, minimal sample handling, and straightforward operation contribute to high efficiency, enabling multiple samples to be processed in a short period without compromising accuracy. The method’s compatibility with automated spectral collection and multivariate data analysis further enhances its practicality, demonstrating that it can be readily integrated into routine analytical workflows for herbal medicines [32].

Whiteness Evaluation

Overall sustainability, encompassing environmental, analytical, and practical dimensions, was further evaluated using the RGB-based White Analytical Chemistry (WAC) framework using RGB12 tool. This approach integrates greenness (green cluster), analytical performance (red cluster), and applicability or operational efficiency (blue cluster) to provide a holistic assessment of method sustainability. By combining these complementary aspects, WAC allows a comprehensive evaluation of the ATR-FTIR method beyond conventional greenness metrics, highlighting its robustness, operational efficiency, and minimal environmental impact. The method scored highly across all three dimensions, confirming that it provides a balanced, eco-friendly, and effective solution for routine quality assessment of Kanchanar Guggulu. This holistic evaluation demonstrates the alignment of the proposed workflow with contemporary sustainable analytical practices, making it a reliable and forward-looking approach for quality assurance in traditional, complementary, and integrative medicine (TCIM) [33].

The combined use of ATR-FTIR spectroscopy, chemometric techniques, and AI-assisted pattern recognition creates a highly effective analytical approach for investigating complex herbal formulations. In comparison with traditional chromatographic methods such as HPLC, LC–MS, or NMR spectroscopy, this approach offers several practical benefits. It enables faster analysis while requiring very little sample preparation, and the measurements can be performed in a non-destructive manner. In addition, the method reduces operational costs and provides highly reproducible results, making it suitable for routine analytical applications.

The sustainability assessment carried out in this study further demonstrated that the developed analytical workflow is consistent with the modern principles of green and sustainable analytical chemistry. Overall, the findings indicate that ATR-FTIR spectroscopy combined with chemometric analysis can function as a rapid and reliable screening tool for the quality evaluation of Ayurvedic medicines. This approach also contributes to strengthening the scientific basis and validation of traditional, complementary, and integrative medicine (TCIM).

Discussion

The integration of ATR-FTIR spectroscopy with chemometric and machine-learning approaches represents an emerging analytical strategy within Traditional, Complementary, and Integrative Medicine research. Such AI-enabled analytical frameworks enable rapid screening of herbal medicines, improve reliability in quality control processes, and support data-driven validation of traditional formulations. Furthermore, these computational tools facilitate objective interpretation of complex phytochemical patterns and provide a scalable approach for routine monitoring of herbal product authenticity in pharmaceutical and nutraceutical industries.

Beyond the unsupervised chemometric analysis performed in this study, the integration of advanced Artificial Intelligence algorithms presents a promising direction for improving the analytical evaluation of complex herbal formulations. Spectral datasets generated through Attenuated Total Reflection Fourier

Transform Infrared Spectroscopy contain extensive multivariate information reflecting the collective chemical profile of polyherbal medicines such as Kanchanar Guggulu. While Principal Component Analysis (PCA) effectively reveals clustering patterns and highlights compositional variability among samples, supervised machine learning approaches can further enhance predictive accuracy and automated classification.

In future work, algorithms such as Support Vector Machine, Random Forest, and Artificial Neural Network could be integrated with ATR-FTIR spectral fingerprints to construct robust predictive models for authentication and quality grading of herbal medicines. These algorithms are capable of identifying subtle nonlinear relationships within complex spectral datasets and can significantly improve discrimination between authentic formulations and adulterated or substandard products. Additionally, machine learning models can be trained using spectral libraries derived from validated reference samples, enabling automated recognition systems for large-scale quality monitoring of traditional medicines.

The application of such AI-enabled analytical platforms holds particular promise for advancing research in Traditional, Complementary, and Integrative Medicine, where herbal formulations often contain multiple bioactive constituents and exhibit inherent variability due to differences in plant origin, processing methods, and storage conditions. By integrating rapid spectroscopic techniques with predictive machine learning algorithms, future analytical frameworks could enable real-time quality assessment, improved reproducibility, and standardized evaluation of Ayurvedic formulations. This convergence of spectroscopy, chemometrics, and artificial intelligence represents an emerging paradigm that can significantly strengthen the scientific validation and global acceptance of traditional herbal medicines.

Conclusions

Traditional Ayurvedic formulations such as Kanchanar Guggulu are widely used for managing various chronic health conditions within the framework of Traditional, Complementary, and Integrative Medicine. However, variations in raw materials, manufacturing processes, and sourcing practices can sometimes lead to inconsistencies in product quality across different commercial preparations. Ensuring the authenticity and uniformity of such traditional formulations is therefore essential for maintaining their therapeutic reliability and global acceptance.

In the present work, a rapid and reliable analytical strategy based on Attenuated Total Reflection Fourier Transform Infrared Spectroscopy was developed for the quality evaluation of Kanchanar Guggulu formulations. Compared with conventional chromatographic and advanced spectroscopic methods that often require complex sample preparation, costly instrumentation, and time-consuming procedures, the proposed ATR-FTIR approach offers a simpler, faster, and non-destructive alternative. The method enables direct spectral fingerprinting of samples, allowing efficient characterization of their overall chemical profile.

To enhance the interpretability of spectral data, multivariate analysis using Principal Component Analysis was applied. This chemometric approach effectively differentiated commercial samples and highlighted variations in their spectral patterns when compared with the authenticated reference formulation. The integration of spectroscopic techniques with chemometric data analysis reflects the growing role of Artificial Intelligence in modern analytical frameworks, where machine-assisted interpretation of complex datasets can support the scientific validation and quality control of traditional herbal medicines.

An additional strength of the developed method lies in its environmental sustainability. The analytical procedure was designed to minimize ecological impact by eliminating the use of hazardous organic solvents and reducing sample preparation steps. To systematically assess the environmental friendliness of the approach, multiple analytical sustainability metrics—including AGREE, Complex GAPI, BAGI, and RGB12 tool—were applied. The high scores obtained through these assessment tools demonstrate that the developed ATR-FTIR method aligns well with contemporary principles of green and sustainable analytical chemistry.

Overall, the proposed AI-assisted spectroscopic strategy provides a rapid, cost-effective, and environmentally responsible solution for the routine quality assessment of Kanchanar Guggulu formulations. By combining modern spectroscopic analysis, chemometric data interpretation, and sustainability evaluation, this study contributes to strengthening the scientific framework for quality control within traditional medicine systems. Such integrative analytical approaches can play an important role in supporting the modernization, standardization, and global acceptance of Ayurvedic formulations within the expanding field of TCIM.

Declarations

Competing interests

The authors declare no competing interests

Human Ethics and Consent to Participate declarations

Not applicable

Funding Declaration

Not applicable

Author Contribution

Rinkal: writing – Original draft, methodology, Ai-driven chemometrics analysis, Sustainability assessment, Authentication.Kunjal: Validation, administration.Shailvi: Formal analysis, investigation.Nikunj: writing – Review & editing, supervision, Concept.

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Table

Table 3 is available in the Supplementary Files section.

Figures

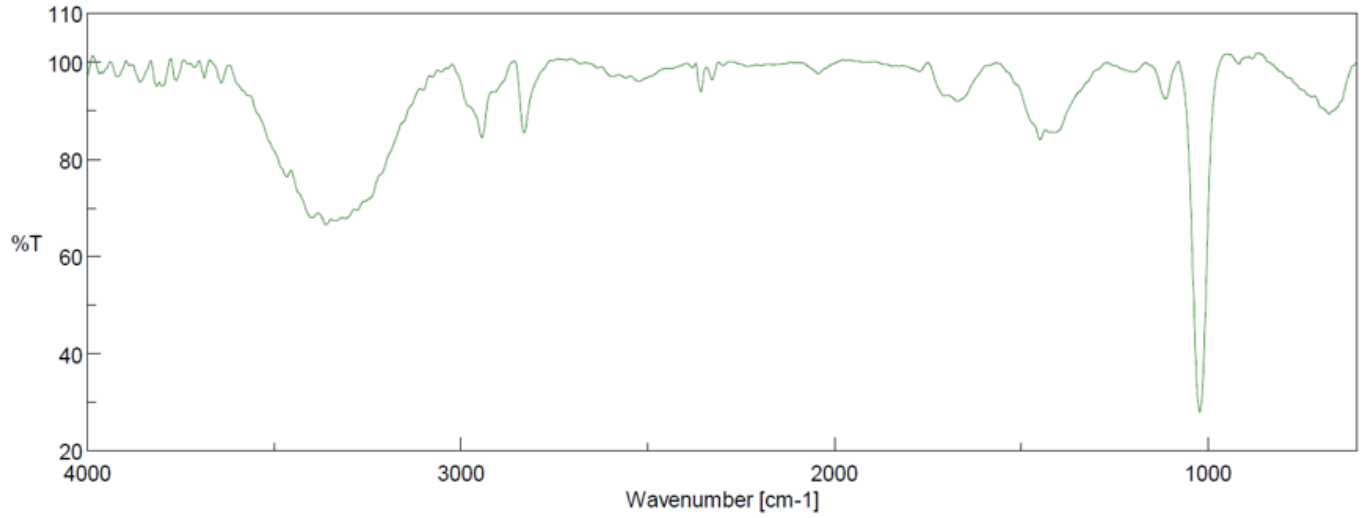


Figure 1

ATR-FTIR spectrum of authenticated in-house Kanchanar Guggulu sample showing characteristic functional group bands.

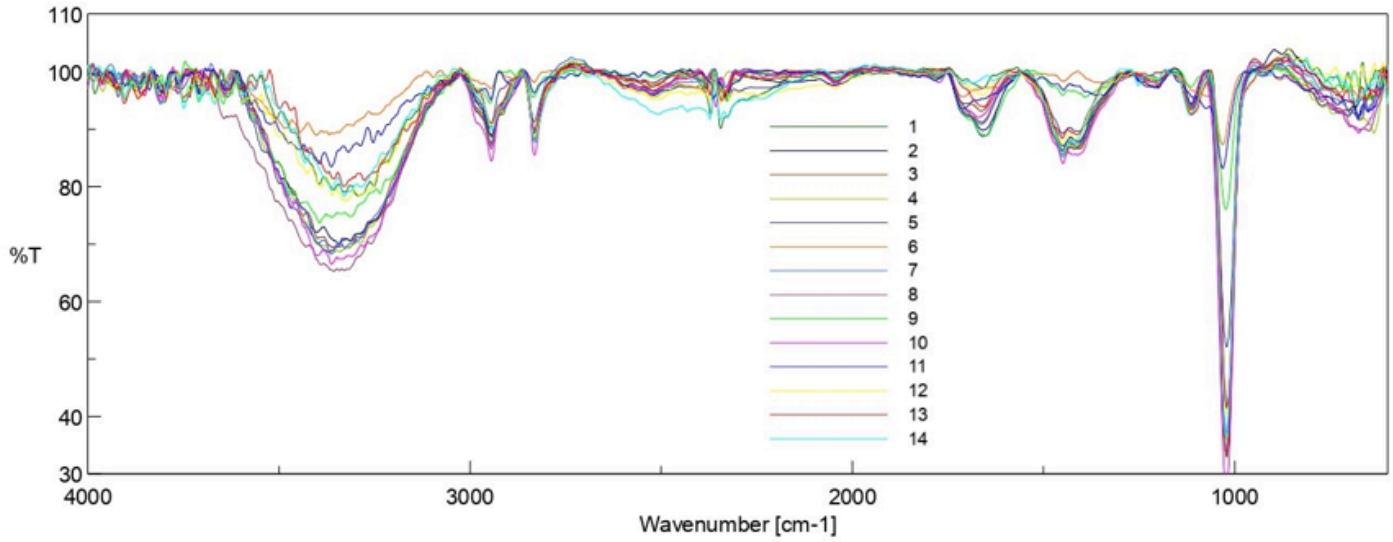


Figure 2

Overlay ATR-FTIR spectra of thirteen Kanchanar Guggulu samples illustrating variations in spectral fingerprints among commercial formulations.

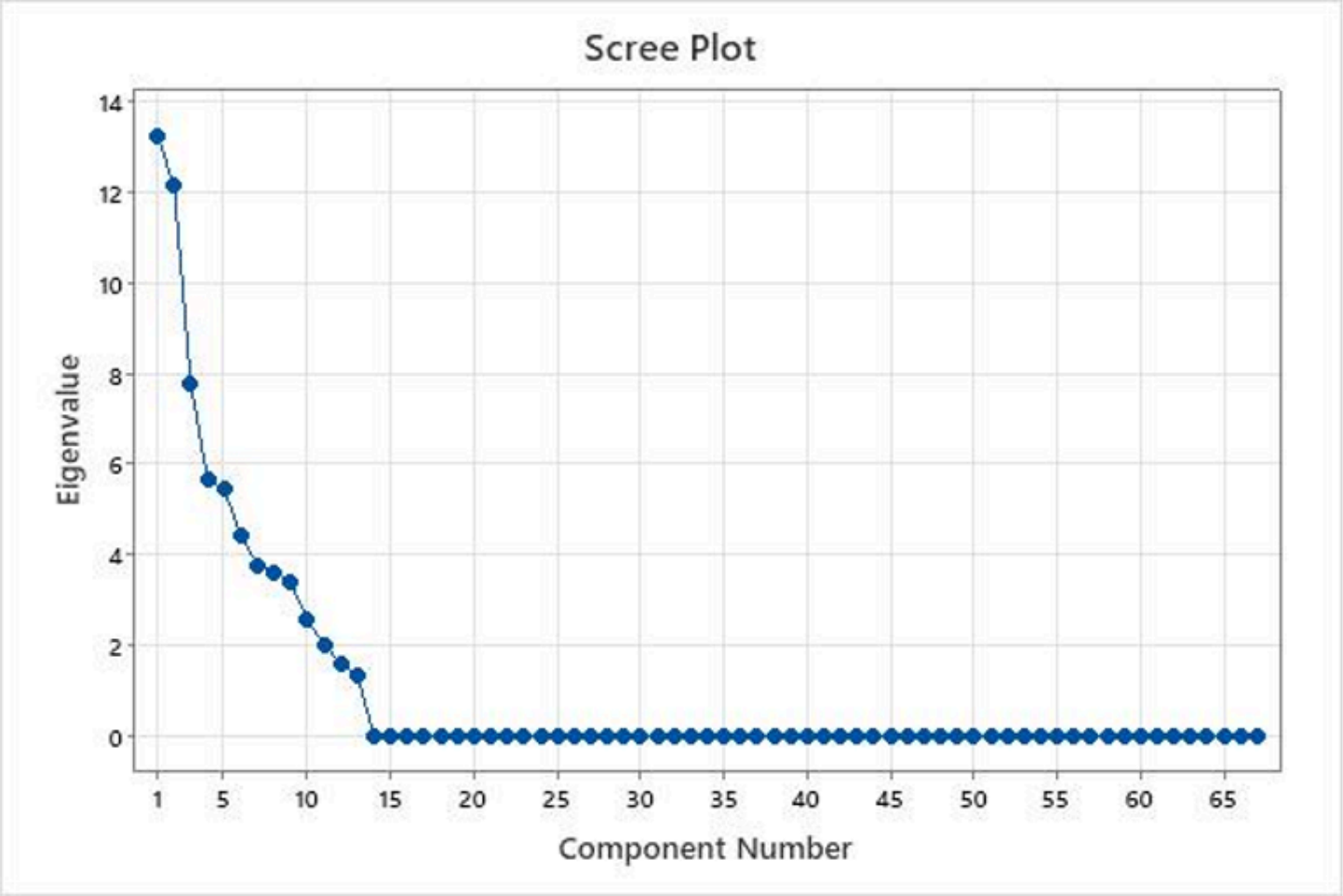


Figure 3

Scree plot showing variance explained by principal components derived from ATR-FTIR spectral data.

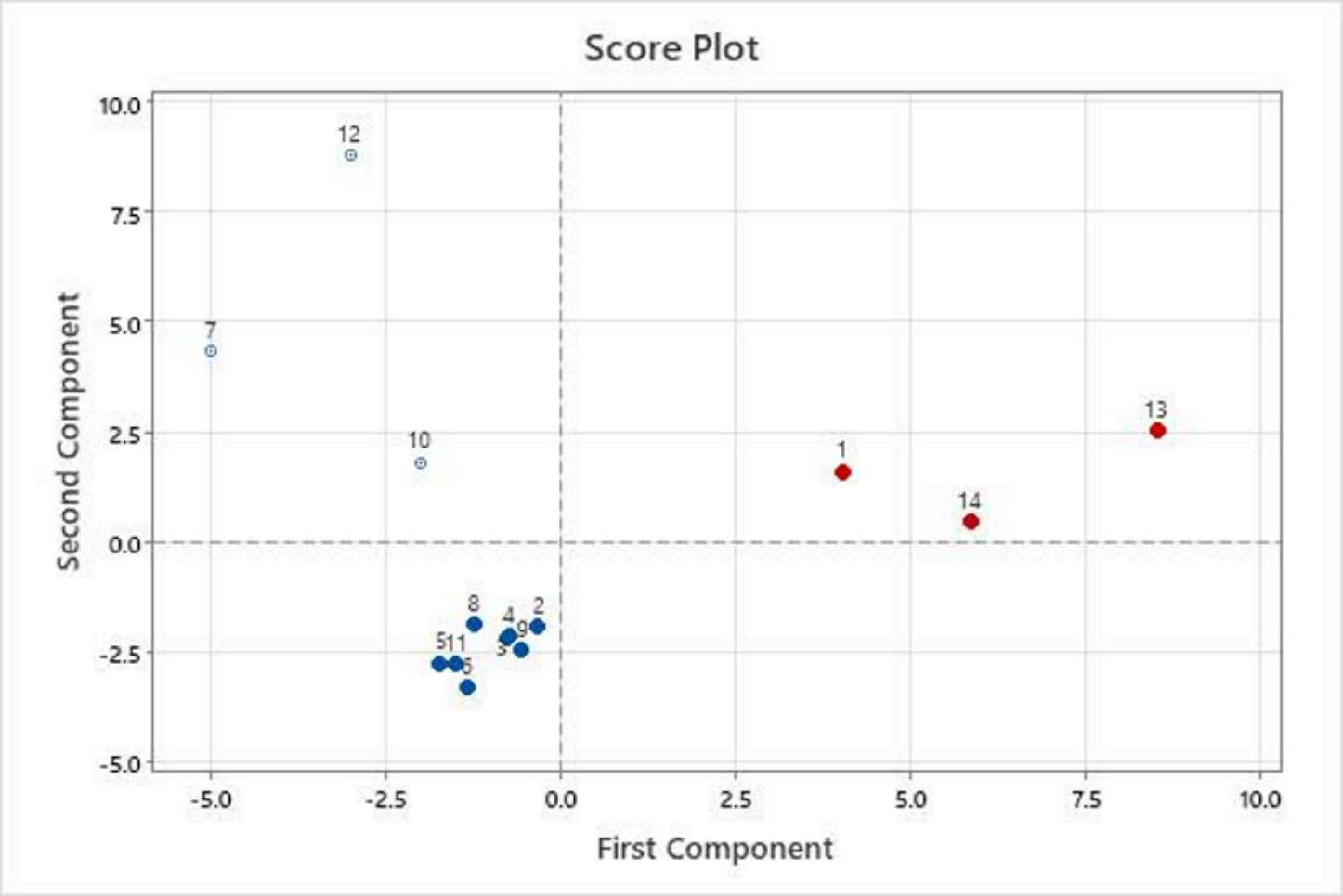


Figure 4

PCA score plot showing clustering pattern of Kanchanar Guggulu samples based on ATR-FTIR spectral fingerprints.

Supplementary Files

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- Table3.docx