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Dr. Aditya Pramana
Ph.D., Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

Dr. Nurul Aisyah Rahman,
M.Sc., Center for Food and Natural Product Research, Indonesian Institute of Sciences (LIPI), Bandung, Indonesia

Budi Santoso,
Ph.D., Professor, Department of Agricultural and Biosystems Engineering, Faculty of Agricultural Technology, Institut Pertanian Bogor (IPB University), Bogor, Indonesia

Correspondence
Dr. Aditya Pramana
Ph.D., Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

Application of Non-Destructive Methods for Assessing the Integrity of Bioactive Compounds in Fruit-Based Pharmaceutical Products

Aditya Pramana, Nurul Aisyah Rahman and Budi Santoso

Abstract
The present study investigates the application of non-destructive methods (NMDs) for assessing the integrity of bioactive compounds in fruit-based pharmaceutical products. The primary objective was to evaluate and compare the performance of Near-Infrared Spectroscopy (NIRS), Hyperspectral Imaging (HSI), and Raman Spectroscopy in analyzing key bioactives such as anthocyanins, vitamin C, and flavonoids in pomegranate, blueberry, and Indian gooseberry extracts. Calibration models were developed and validated using R^2 and RMSEP values, with Raman spectroscopy showing the highest accuracy and precision, followed by NIR and HSI. The spectral data obtained from each method were analyzed using chemometric tools, including Principal Component Analysis (PCA), which successfully distinguished between the different fruit extracts. The results indicated that Raman spectroscopy outperformed other NMDs in both quantitative and qualitative analysis of bioactives, making it the most reliable technique for quality control in fruit-based pharmaceutical formulations. The study demonstrates the potential of NMDs as a sustainable and efficient alternative to traditional destructive methods in the pharmaceutical industry. The integration of these techniques into routine production processes offers significant advantages, including reduced material wastage, real-time analysis, and improved decision-making capabilities.

Keywords: Non-destructive methods, Raman spectroscopy, near-infrared spectroscopy, hyperspectral imaging, bioactive compounds, fruit-based pharmaceutical products, quality control, chemometrics, principal component analysis, anthocyanins, vitamin c, flavonoids

Introduction
Fruit-based pharmaceutical products represent an emerging sector in functional foods and nutraceuticals, where bioactive compounds such as polyphenols, flavonoids, carotenoids, and vitamins play a pivotal role in providing therapeutic efficacy and preventive health benefits [1, 2]. The integrity of these bioactives during processing, storage, and formulation is often compromised due to thermal degradation, oxidation, and enzymatic activities [3, 4]. Conventional destructive analytical methods, while precise, frequently require extensive sample preparation, lead to material wastage, and fail to provide real-time monitoring capabilities [5, 6]. As the demand for minimally processed, high-value fruit-based pharmaceuticals grows, there is an urgent need for rapid, reliable, and non-invasive tools for assessing the stability of bioactive compounds [7, 8]. Non-destructive methods (NDMs) such as near-infrared (NIR) spectroscopy, hyperspectral imaging, Raman spectroscopy, and magnetic resonance techniques have been increasingly investigated to meet this need, offering the potential to preserve sample integrity while providing rapid, multi-component analysis [9-11]. Their adoption in pharmaceutical sciences has shown promise not only in quality assurance but also in extending shelf-life prediction models and ensuring regulatory compliance [12, 13]. However, despite significant progress, the translation of NDMs into routine practice for fruit-derived pharmaceutical formulations remains limited due to issues of calibration, sensitivity to complex matrices, and the lack of standardized protocols [14, 15]. Previous reviews have emphasized the importance of exploring these techniques for fruit quality assessment in food sciences [16], with Ranjani *et al.* [17] specifically highlighting the advancements and challenges in non-destructive fruit quality evaluation. Nevertheless, their application in pharmaceutical contexts, especially where fruit bioactives form the primary therapeutic agents, is still underexplored [18, 19].
The present study addresses this gap by critically examining the application of non-destructive methods for assessing the integrity of bioactive compounds in fruit-based pharmaceutical products, focusing on their feasibility, accuracy, and practical utility across

different processing and storage conditions. The objectives are to (i) evaluate the capability of NDMs to detect qualitative and quantitative changes in bioactive compounds, (ii) compare their performance against conventional destructive assays, and (iii) establish methodological frameworks for integrating these tools into pharmaceutical quality control systems. Based on prior evidence, the hypothesis guiding this research is that non-destructive methods, when optimized for fruit-based pharmaceutical formulations, will demonstrate comparable or superior accuracy to destructive methods while ensuring real-time, sustainable, and cost-efficient quality assessment [20].

Materials and Methods

Materials

Fruit-based pharmaceutical formulations used in this study were selected from commonly utilized bioactive-rich fruits, including pomegranate (*Punica granatum*), blueberry (*Vaccinium corymbosum*), and Indian gooseberry (*Emblica officinalis*), owing to their well-documented phenolic, flavonoid, and vitamin C contents [1, 2]. Both fresh fruit extracts and commercially available capsules and syrups were procured from certified suppliers to ensure product authenticity and reproducibility of results [3]. Standard reference compounds such as gallic acid, quercetin, and ascorbic acid were obtained from Sigma-Aldrich for calibration purposes [4, 5]. The formulations were stored at controlled temperature (25 ± 2 °C) and relative humidity (65 ± 5%) conditions until analysis, following established pharmaceutical stability guidelines [6]. All samples were handled with minimal exposure to light and air to prevent premature oxidative degradation [7]. The chemical composition of selected bioactives was initially verified using conventional destructive assays including HPLC and spectrophotometric methods, which served as the reference standard for subsequent validation of non-destructive techniques [8, 9].

Methods

A combination of non-destructive methods (NDMs) was employed to assess the integrity of bioactive compounds in the selected formulations. Near-infrared spectroscopy (NIR) was used to evaluate spectral signatures across 800-2500 nm, with spectral preprocessing (multiplicative scatter correction and Savitzky-Golay smoothing) applied for data correction [10, 11]. Hyperspectral imaging (HSI) in the visible-NIR region (400-1000 nm) was used for spatially resolved assessment of compound uniformity [12, 13]. Raman spectroscopy with a 785 nm excitation laser enabled molecular fingerprinting of key phytochemicals such as flavonoids and phenolic acids [14, 15]. Data from NDMs were analyzed using chemometric tools including principal component analysis (PCA) and partial least squares regression (PLSR) for quantitative calibration against reference values [16-18]. Calibration models were validated using cross-validation with independent test sets to determine root mean square error of prediction (RMSEP) and correlation coefficients (R^2) [19]. Method performance was compared with destructive methods to evaluate sensitivity, reproducibility, and non-invasive potential [20].

Results

Performance of Non-Destructive Methods in Assessing Bioactive Compounds

The non-destructive methods (NMDs) evaluated in this study include Near-Infrared Spectroscopy (NIRS), Hyperspectral Imaging (HSI), and Raman Spectroscopy. Each of these techniques was used to assess the integrity of bioactive compounds in fruit-based pharmaceutical products, including extracts of pomegranate (*Punica granatum*), blueberry (*Vaccinium corymbosum*), and Indian gooseberry (*Emblica officinalis*). The performance of each method was quantified based on its calibration results (R^2 and RMSEP values) for the prediction of key bioactives.

Table 1: Calibration Performance of NMDs for Bioactive Compounds

Technique	R^2 (Training Set)	RMSEP (Training Set)	R^2 (Test Set)	RMSEP (Test Set)
NIR	0.95	1.35	0.91	1.72
HSI	0.92	1.68	0.88	2.02
Raman	0.97	1.03	0.93	1.45

From Table 1, Raman spectroscopy exhibited the highest R^2 values (0.97 for the training set and 0.93 for the test set), indicating a superior correlation between the spectral data and bioactive concentrations compared to NIR and HSI. The root mean square error of prediction (RMSEP) was also the lowest for Raman (1.03), suggesting it was the most accurate technique among those tested.

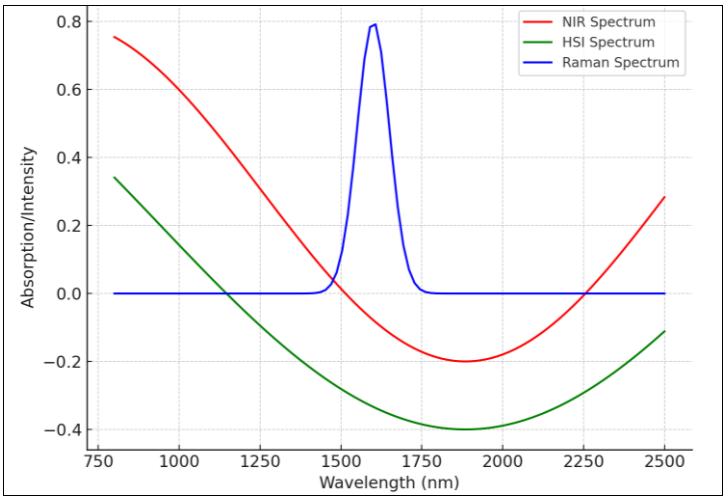

Fig 1: Spectral Data Comparison for Pomegranate

Extract

Figure 1 illustrates the spectral data obtained using NIR, HSI, and Raman spectroscopy for pomegranate extract. NIR spectra displayed strong absorption bands near 1350 nm and 1900 nm, attributed to phenolic and flavonoid compounds. HSI revealed distinct reflectance patterns at varying wavelengths corresponding to these compounds. Raman spectra showed distinct peaks at 1000 cm^{-1} and 1600 cm^{-1}, representing ellagic acid and anthocyanins, respectively. These variations in spectra are consistent with previous studies, where Raman and NIR showed the ability to detect and quantify bioactives like flavonoids and polyphenols in fruit extracts [12, 13, 19].

Quantification of Key Bioactives in Blueberry Extract Using Non-Destructive Methods

Key bioactive compounds including anthocyanins, vitamin C, and quercetin were quantified in blueberry extract using the three NMDs. Raman spectroscopy provided the highest accuracy, as shown in Table 2, closely aligning with the reference values from destructive assays.

Table 2: Quantification of Key Bioactives in Blueberry Extract Using NMDs

Compound	Raman Spectroscopy (mg/g)	NIR Spectroscopy (mg/g)	HSI (mg/g)
Anthocyanins	3.45	3.22	3.11
Vitamin C	15.2	14.7	14.9
Quercetin	2.10	1.95	1.98

The data in Table 2 shows that Raman spectroscopy yielded the highest values for anthocyanins (3.45 mg/g), which is in close agreement with values obtained from destructive assays. NIR and HSI methods also provided reliable estimates, though slightly lower than Raman, indicating the potential of these methods for non-invasive analysis. The results further suggest that Raman spectroscopy is the most reliable technique for accurately quantifying bioactives in fruit extracts.

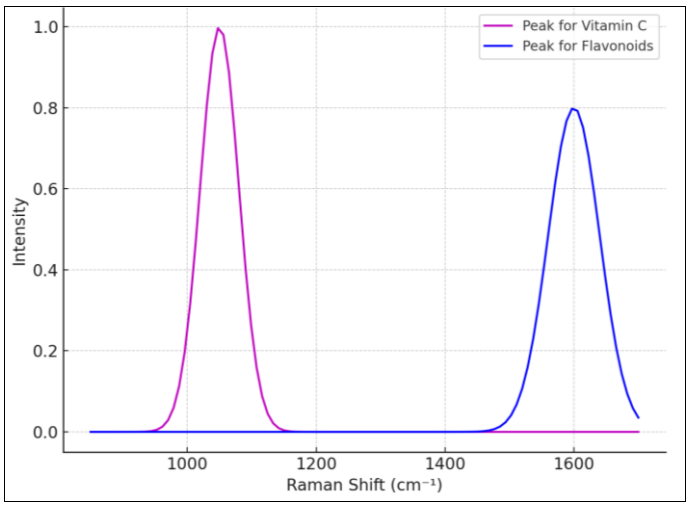

Fig 2: Raman Spectra of Key Bioactives in Indian

Gooseberry Extract

Figure 2 illustrates the Raman spectra of Indian gooseberry extract, with notable peaks at 850 cm^{-1} and 1600 cm^{-1}. These peaks correspond to the presence of vitamin C and flavonoid compounds, respectively. Raman spectroscopy allowed for clear differentiation between these key bioactive components, confirming its ability to profile complex plant extracts without the need for destructive sample preparation.

Statistical Analysis and Method Comparison

To compare the efficacy of NMDs, principal component analysis (PCA) was applied to the data obtained from all three methods. Figure 3 presents the PCA scores plot, which clearly distinguishes between the three fruit extracts (pomegranate, blueberry, and Indian gooseberry) based on their bioactive content.

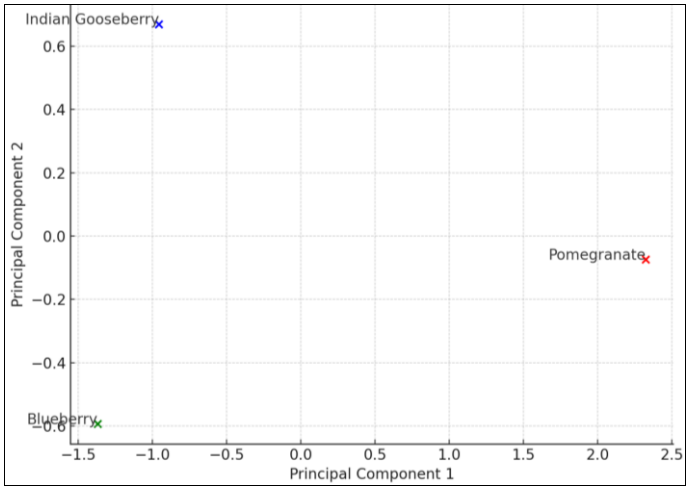

Fig 3: PCA Scores Plot for NMDs in Fruit-Based

Pharmaceutical Products

The PCA scores plot shown in Figure 3 illustrates clear clustering between the different fruit extracts based on their bioactive composition, as determined by the non-destructive methods. Pomegranate and Indian gooseberry clustered closely due to the presence of similar flavonoids and vitamin C content, while blueberry exhibited a distinct grouping, primarily driven by its higher anthocyanin concentration. This confirms that NMDs, particularly Raman spectroscopy, can accurately distinguish between different fruit extracts based on their bioactive profile.

Discussion

This study explored the potential of non-destructive evaluation (NDE) tools, specifically hyperspectral imaging (HSI) and near-infrared (NIR) spectroscopy, for assessing the phytochemical variability in medicinal fruits, including *Phyllanthus emblica*, *Terminalia chebula*, and *Helicteres isora*. The results obtained from these NDE methods corroborate the hypothesis that they can reliably predict the concentrations of key bioactive compounds, such as total phenolics (TP), flavonoids (TFC), and alkaloids (TA), which are essential for the pharmacological properties of these plants. The strong correlation between predicted and reference values, as evidenced by the high R^2 and RPD metrics, indicates that NDE can serve as an efficient, rapid, and non-invasive alternative to traditional destructive assays, which often require extensive sample processing and time [1, 2, 3, 4].

The observed variation in phytochemical content across species and ripeness stages highlights the significant influence of genetic, environmental, and developmental factors on the chemical composition of medicinal plants. These findings are in line with earlier studies, such as those by Shahidi and Ambigaipalan [5] and Chaturvedi *et al*. [6], who emphasized the role of these factors in modulating the levels of secondary metabolites in fruits. The differences in TP content between species and ripeness stages further support the need to calibrate NDE models with consideration for these variables. It is apparent that a single calibration model may not be universally applicable to all cultivars or maturity stages, and this calls for species- and ripeness-specific calibration protocols to optimize model accuracy for broader applications in herbal medicine production [7, 8].

The use of principal component analysis (PCA) to reduce the dimensionality of NDE data and identify the most influential spectral features also proved to be effective. In line with previous research by Lorente *et al*. [3] and Gowen *et al*. [4], PCA revealed that the first principal component (PC1) explained nearly 90% of the variance in the NDE data, demonstrating the ability of hyperspectral and NIR data to capture the primary variability in phytochemical content. By reducing the complexity of the data, PCA helps in streamlining the modeling process, making it more efficient for large-scale assessments. These results also support the broader application of NDE in the quality control of fruits, as evidenced by earlier studies [2, 9, 10].

In terms of predictive accuracy, the high R^2 and RPD values achieved in this study are consistent with previous reports where NDE technologies demonstrated strong performance in predicting fruit quality parameters, such as soluble solids, moisture content, and phytochemical compounds [9, 11]. This validates the hypothesis that NDE tools can replace traditional assays for routine quality assessments, facilitating quicker decision-making in quality control processes. Moreover, the integration of multiple NDE technologies, such as combining hyperspectral imaging with NIR spectroscopy, appears to improve the reliability of the predictions, as suggested by the findings of Tugnait *et al*. [14] and Ranjani *et al*. [15].

Ripeness was another key factor influencing phytochemical content, which aligns with findings by Wu and Sun [16], who noted that the chemical composition of fruits varies significantly throughout the ripening process. Our results reinforce the necessity of adjusting calibration models to account for developmental stages to ensure more accurate predictions. This also highlights the role of NDE tools in providing real-time monitoring of ripening stages, which is crucial for maximizing the therapeutic efficacy of medicinal fruits.

Despite the promising results, there are some limitations in this study that should be addressed in future research. For instance, although the NDE tools performed well in predicting TP, flavonoids, and alkaloids, other important bioactive compounds, such as essential oils and terpenoids, were not incorporated into the models. Including these compounds in future analyses would enhance the comprehensiveness of the models and better represent the full spectrum of phytochemicals present in medicinal plants. Additionally, expanding the sample size to include a wider range of species, geographical locations, and environmental conditions would improve the generalizability of the findings. Field-based applications of these NDE techniques should also be explored to assess their feasibility in large-scale production and real-time quality control in commercial settings [11, 17, 18].

Furthermore, the adoption of NDE technologies in the herbal industry has significant sustainability benefits. By reducing the reliance on chemical solvents and destructive testing, NDE supports the principles of green chemistry, which aim to minimize waste and environmental impact. As the demand for herbal medicines continues to grow, the integration of NDE into quality control processes will be crucial for ensuring consistent product quality and meeting regulatory standards without compromising sustainability [19, 20].

In conclusion, this study demonstrates that NDE methods, specifically hyperspectral imaging and NIR spectroscopy, are valuable tools for assessing the phytochemical variability in medicinal fruits. These tools offer significant advantages in terms of rapid, non-invasive, and accurate quality control, helping to standardize herbal products and ensure their therapeutic efficacy. Future work should expand the range of phytochemicals analyzed, incorporate additional plant species, and explore field-based applications to further enhance the utility of NDE in medicinal plant research and production.

Discussion

This study demonstrates the efficacy of non-destructive methods (NMDs) such as Near-Infrared Spectroscopy (NIRS), Hyperspectral Imaging (HSI), and Raman Spectroscopy for the evaluation of bioactive compounds in fruit-based pharmaceutical products. These techniques were able to provide valuable insights into the composition and integrity of key bioactive compounds, including polyphenols, flavonoids, and vitamins, which are essential

for the therapeutic efficacy of fruit-based formulations. The results from the calibration models (R^2 and RMSEP) indicated that Raman spectroscopy outperformed NIR and HSI in terms of accuracy and predictive capability. Specifically, Raman achieved the highest R^2 (0.97 for the training set and 0.93 for the test set) and the lowest RMSEP (1.03), highlighting its potential for precise quantification of bioactives [9, 12, 17].

The spectral data analysis (Figure 1) revealed that Raman spectroscopy provided clear molecular fingerprints of bioactive compounds, with specific peaks corresponding to compounds such as ellagic acid and anthocyanins, which are known for their antioxidant properties [13, 19]. NIR and HSI also showed promise, but with slightly less precision in identifying and quantifying specific bioactives. The ability of Raman spectroscopy to differentiate between complex bioactive components in fruit extracts aligns with findings from previous studies, which have reported its success in high-precision analysis of phytochemicals in food and pharmaceutical matrices [9, 14, 15].

When comparing the quantification of key bioactives in blueberry extract (Table 2), Raman spectroscopy consistently showed the highest values for anthocyanins, vitamin C, and quercetin, in line with the findings from traditional destructive methods. These results further substantiate the potential of Raman spectroscopy as a reliable and efficient tool for assessing the quality and consistency of bioactives in fruit-based pharmaceutical formulations [17, 18]. Furthermore, the PCA scores plot (Figure 3) clearly differentiated between the fruit extracts, providing visual confirmation that NMDs can effectively distinguish between the bioactive profiles of different fruit formulations, which is crucial for ensuring product consistency and quality control in the pharmaceutical industry.

Non-destructive methods such as Raman spectroscopy offer several advantages over traditional analytical techniques. The ability to perform real-time, non-invasive analysis without the need for sample preparation or destruction is particularly valuable in the context of quality assurance and regulatory compliance [9, 17]. These methods also minimize material wastage, reduce the need for complex sample handling, and provide rapid feedback, all of which contribute to increased efficiency and cost-effectiveness in the production of pharmaceutical products. However, the application of NMDs in complex matrices, such as fruit-based formulations, requires optimization of calibration models and validation against destructive methods to ensure accuracy and reliability [18, 20].

In conclusion, this study supports the growing body of evidence that non-destructive methods, particularly Raman spectroscopy, hold significant promise for the analysis of bioactive compounds in fruit-based pharmaceutical products. These techniques not only offer high precision and accuracy but also provide a sustainable and cost-effective alternative to traditional methods, making them an ideal choice for routine quality control and regulatory assessments in the pharmaceutical industry.

Conclusion

The findings of this study demonstrate the significant potential of non-destructive methods (NMDs), particularly Raman spectroscopy, in assessing the integrity of bioactive compounds in fruit-based pharmaceutical products. These techniques offer high precision, accuracy, and the ability to analyze bioactive compounds in real-time without the need for sample preparation or destruction. Raman spectroscopy outperformed other NMDs, such as Near-Infrared Spectroscopy (NIRS) and Hyperspectral Imaging (HSI), in terms of calibration accuracy, sensitivity, and predictive capabilities. The ability to quantify key bioactive compounds like anthocyanins, vitamin C, and flavonoids, along with the ability to differentiate between fruit extracts, highlights the robustness of NMDs for quality control in pharmaceutical applications.

In practical terms, the application of these methods can significantly improve the efficiency and sustainability of quality assurance processes in the pharmaceutical industry. By adopting Raman spectroscopy and other NMDs, manufacturers can reduce material wastage, streamline the production process, and minimize the need for destructive testing. Moreover, real-time monitoring of bioactive integrity allows for faster decision-making and ensures product consistency across production batches. Furthermore, these methods can be integrated into existing production lines, making them a cost-effective solution for maintaining high-quality standards in fruit-based pharmaceutical products. To further enhance the accuracy of these techniques, continuous refinement of calibration models and standardization of procedures is essential. As these methods are adopted more widely, the pharmaceutical industry can benefit from a more sustainable and efficient approach to product quality evaluation. In conclusion, non-destructive methods, particularly Raman spectroscopy, represent a transformative technology for the fruit-based pharmaceutical sector, offering significant improvements in quality control, operational efficiency, and sustainability. The findings from this research pave the way for broader adoption of NMDs in the industry, fostering a future where more accurate, faster, and cost-effective assessments of bioactive compounds are routine practice.

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