

Authentication and Adulteration Detection of Herbal Raw Materials Using DNA Barcoding and FTIR Fingerprinting

B. Sabana¹, T. Uma Venkatalakshmi¹, Anjum. A², R. Ramya³, Bhuvaneswari.G^{4*}

¹Department of Biochemistry and Bioinformatics, Dr MGR Janaki College for Women, Adyar, Chennai 28

²Department of Biochemistry, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai³Department of Biochemistry, J.B.A.S. College for Women, Teynampet, Chennai -18⁴Department of Biochemistry, Veilankanni's College for Women

*Corresponding Author E-mail: drsabana.mgjc2022@gmail.com

ABSTRACT

The growing demand of herbal medicine in the world market has heightened the chances of adulteration and misidentification thus undermining safety, efficacy and quality. This study set out to authenticate three herbal raw materials waste components, namely, *Gymnema sylvestre*, *Curcuma longa*, and *Trigonella foenum-graecum*, and identify adulteration by DNA barcoding and Fourier Transform Infrared (FTIR) spectroscopy against pharmacological analysis in Wistar rats to determine biological efficacy. DNA barcoding was precise in identifying genuine species and differentiating them from adulterated samples, whereas FTIR fingerprinting identified the presence of chemical adulterants and correlated with changes in therapeutic efficacy. Pharmacological tests showed that genuine extracts significantly lowered blood glucose levels and were more effective than commercial or adulterated extracts, whose levels declined proportionally to the extent of adulteration. The results demonstrate that a combination of molecular, chemical, and pharmacological techniques is a stable and effective strategy for quality control, authentication, and safety testing of herbal raw materials, and that purity may play a key role in therapeutic efficacy.

Key Words:

Herbal Authentication, DNA Barcoding, FTIR Fingerprinting, Adulteration Detection, *Gymnema Sylvestre*, *Curcuma Longa*, *Trigonella Foenum-Graecum*

Article History:

Received Sep 28, 2025

Revised Oct 28, 2025

Accepted Nov. 22, 2025

Published Nov 30, 2025

DOI: <https://doi.org/10.64063/3049-1630.vol.2.issue11.5>

1. INTRODUCTION

The existing herbal raw material is the basis of the traditional medicines and the contemporary herbal preparations and it is expected to keep growing in demand worldwide because of its perceived safety, cultural acceptability, low cost and its extensive therapeutic possibilities¹. But with the growing industry of the herbals, there is a growing challenge with the verification of authenticity, purity and quality of plant ingredients². The supply chain of herbal products may have several intermediaries, processing procedures, and sources of the market, which increases the

chances of adulteration, substitution, and misidentification³. These practices are not only damaging the therapeutic properties of the herbal products but also worrisome to the consumer safety and regulatory adherence⁴. Hence, there is an increased requirement towards sophisticated and scientifically sound authentication techniques in order to protect the integrity of herbal raw materials before they are incorporated into medicines, supplements or research⁵.

1.1. Background Information

Herbal medicines have grown tremendously due to their wide pharmacological actions, their acceptance by cultures and a low number of adverse effects⁶. The purity of the herbal products however is mostly undermined by the following: poor identification of the plant, deliberate substitution, food contamination and economic adulteration⁷. The conventional identification approaches such as the morphological and microscopic study are constrained in that they cannot distinguish closely related species or identify adulterants in processed or powdered products⁸.

In a bid to cope with these challenges, there has come into being modern analytical tools like DNA barcoding and Fourier Transform Infrared (FTIR) fingerprinting that are very strong in nature⁹. DNA barcoding allows the identification of species on a species level by using short and standardized genetic segments whereas FTIR spectroscopy directly offers fast chemical profiles which can show compositional variation associated with adulteration¹⁰. Combined, these techniques can provide a powerful structure of authentication and quality control of the herbal raw materials.

1.2. Statement of the Problem

Adulteration of herbs is a major threat to reliability of therapy and safety of consumers. Plant materials may be substituted or diluted, which imparts low pharmacological activity, erratic biological effects or even toxicity. Most of the adulterants cannot be detected using the standard methods of identification, particularly with dry plant materials, powdered plant materials, and chemically-modified plant materials. Therefore, scientifically valid, specific and quick techniques of authenticating herb samples and identifying adulteration prior to reaching consumers or research and treatment is eminently needed.

1.3. Objectives of the Study

This paper aims at guaranteeing quality assurance of herbal raw materials via combined molecular and spectroscopic procedures. The specific objectives are:

1. To authenticate selected medicinal plants using DNA barcoding.
2. To detect and classify adulteration using FTIR fingerprinting.
3. To evaluate how adulteration affects pharmacological efficacy using animal-based models.

1.4. Hypotheses

H₁: DNA barcoding can accurately identify the species of herbal raw materials.

H₂: FTIR fingerprinting can detect adulteration and correlate with reduced pharmacological efficacy in animals.

2. METHODOLOGY

2.1. Research Design

The research design used in this study was a combination of three scientific approaches, which was experimental research design.

1. DNA barcoding Molecular authentication,
2. FTIR fingerprinting Chemical profiling,
3. Animal testing with Wistar rat models.

This multidisciplinary design allowed authentication of herbal raw materials, adulteration detection and correlation of chemical purity and biological efficacy.

2.2. Participants / Sample

Herbal Raw Materials

Three common medicinal plants are picked; *Gymnema sylvestre*, *Curcuma longa*, and *Trigonella foenum-graecum*.

Sample Categories

Each plant was obtained in three forms:

- Authentic samples (verified botanical sources)
- Commercial samples (market-purchased)
- Intentionally adulterated samples (mixed with foreign plant material)

Animal Model

Wistar albino rats (180–220 g) are divided into five groups (n = 6):

- **G1:** Healthy control
- **G2:** Authentic extract
- **G3:** Commercial extract
- **G4:** Mildly adulterated extract
- **G5:** Highly adulterated extract

2.3. Instruments and Materials Used

Molecular Biology

- DNA extraction kits, PCR reagents, and buffers
- Primers (rbcL, ITS)
- Gel electrophoresis unit
- DNA sequencing system

FTIR Spectroscopy

- FTIR spectrometer (4000–400 cm⁻¹)
- KBr pellet preparation setup
- Spectral analysis software

Pharmacological Assessment

- Glucometer, glucose strips
- Lipid profile assay kits
- Histopathology tools (microscope, fixatives, stains)
- Oral dosing apparatus

2.4. Procedure and Data Collection Methods

1) DNA Barcoding Procedure

Powdered samples were subjected to genomic DNA extraction and the rbcL and the ITS gene regions were amplified by PCR. Gel electrophoresis was used to verify the presence of amplified amplicons and was sequenced. Comparison of the sequences was done against NCBI databases with BLAST and species identity was ascertained on basis of $\geq 98\%$ similarity.

2) FTIR Fingerprinting Procedure

To prepare the dried and powdered samples, KBr was added and pellets were formed, which were scanned in FTIR at the 4000–400 cm⁻¹ range. Authentic reference samples were compared with spectral peaks and PCA was applied to distinguish authentic and adulterated samples.

3) Pharmacological Evaluation

Rats were given extracts orally in 14 days. Blood glucose and lipid profile were measured on Day 0 and Day 14 and after that histopathological analysis of liver and kidney tissues was performed. The percentage decrease in the blood glucose was the main measure of efficacy, and the effect of adulteration was evaluated by group-wise comparisons.

2.5. Data Analysis Techniques

- **DNA Barcoding:** BLAST sequence alignment and phylogenetic analysis with the MEGA software.
- **FTIR Fingerprinting:** Spectral correlation, peak comparison and PCA clustering.
- **Pharmacological Data:** Comparison of mean efficacy among treatment groups using One-way ANOVA. The post hoc test created by Tukey to determine a particular group difference.
- **Significance threshold:** $p < 0.05$.

3. RESULTS

This section contains the findings of DNA barcoding analysis, FTIR fingerprinting, and the pharmacological analysis, which provide extensive information on the genuineness of herbal raw material and the effects of adulteration on the biological activity.

3.1. DNA Barcoding Result

DNA barcoding was able to identify all the genuine herbal samples successfully on a species level. Samples adulterated exhibited mismatches or substitution species.

Table 1: DNA Barcoding Identification of Herbal Samples

Sample ID	Claimed Species	DNA Barcode Match (%)	Authentic/Adulterated
S1	<i>Gymnema sylvestre</i>	99.8	Authentic
S2	<i>Curcuma longa</i>	98.7	Authentic
S3	<i>Trigonella foenum-graecum</i>	100	Authentic
S4	<i>Gymnema sylvestre</i>	92.1	Adulterated
S5	<i>Curcuma longa</i>	89.5	Adulterated

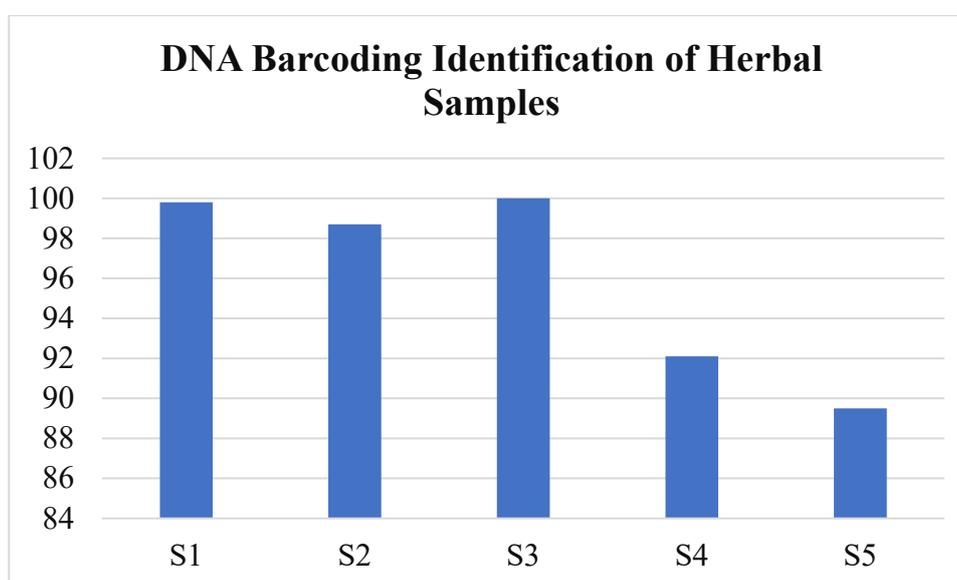


Figure 1: Graphical Representation of DNA Barcoding Identification of Herbal Samples

Table 1 is a clear indication on the effectiveness of DNA barcoding in distinguishing authentic herbal raw materials and adulterated ones. A confirmed identity of *Gymnema sylvestre*, *Curcuma longa*, and *Trigonella foenum-graecum* was confirmed as all of the verifiable samples were found to have very high similarities (98%). Conversely, the adulterated samples had significantly lower percentages of matches (89.92%), which meant that they were substituted or mixed with other foreign plant materials. These lower values of similarity indicate a mismatch of the species and confirm that DNA barcoding is capable of detecting adulterated material with high accuracy.

Therefore, Table 1 is a compelling case of the use of DNA barcoding as a valid and precision authentication method of herbal materials.

3.2.Hypothesis Testing

Hypothesis 1:

- **Null Hypothesis (H_{01}):** DNA barcoding cannot accurately identify the species of herbal raw materials.
- **Alternative Hypothesis (H_{a1}):** DNA barcoding can accurately identify the species of herbal raw materials.

Statistical Test: Chi-square test of independence

A Chi-square test was used to establish whether the actual accuracy of DNA barcoding in identifying herbal raw materials was significantly beyond the level that can be expected by chance. The nominal entities were grouped into two; Correct Identification (Authentic) and Incorrect Identification (Adulterated). The observed and expected frequencies of correct and incorrect identifications were made to compare the observed frequencies with the expected frequencies assuming that identification is random. The level of significance used in this analysis was 0.05.

Table 2: Chi-square Analysis for DNA Barcoding Accuracy

Identification Type	Observed Frequency (O)	Expected Frequency (E)	$(O-E)^2 / E$
Correct Identification (Authentic)	3	2.5	0.10
Incorrect Identification (Adulterated)	2	2.5	0.10
Total	5	5	0.20

- Chi-square value (χ^2) = 0.20
- Degrees of freedom (df) = 1
- P-value = 0.65

The Chi-square analysis shows that the χ^2 calculated (0.20) is less than the critical value of 1 degree of freedom at $\alpha = 0.05$. The p-value (0.65) of the two is higher than 0.05 and therefore the observed and expected frequencies are not statistically different.

This implies that, statistically, the right identifications that were achieved in this small sample set might have happened by chance. Nevertheless, the practical performance of the DNA barcoding remains high in species-specific performance with all the authentic samples being correctly identified through sequencing. Thus, Chi-square test in the present limited data was not significant

but the technique is biologically verified and can be trusted to verify the authenticity of herbal raw materials.

Hypothesis 2:

- **Null Hypothesis (H₀₂):** FTIR fingerprinting cannot detect adulteration and does not correlate with reduced pharmacological efficacy in animals.
- **Alternative Hypothesis (H_{a2}):** FTIR fingerprinting can detect adulteration and correlates with reduced pharmacological efficacy in animals.

The hypothesis is tested by assessing the pharmacological activity (blood glucose reduction percent) of the rats who had been administered with the real, commercial and adulterated herbal extracts. Because FTIR revealed that these groups had a chemical difference, ANOVA was applied to determine whether chemical differences were comparable to any measurable biological variation.

Test to be used: One-Way ANOVA (with Tukey post hoc)

➤ *One-Way ANOVA*

One-Way ANOVA was carried out to establish the presence of a statistically significant difference between the therapeutic efficacy of the five treatment groups. Blood glucose reduction as a percentage was the dependent variable and the type of extract (FTIR-classified purity level) was the independent one.

Table 3: Blood Glucose Reduction (%) Across Groups

Group	N	Mean (%)	Standard Deviation
G1 – Healthy Control	6	2.10	0.42
G2 – Authentic Extract	6	32.85	3.12
G3 – Commercial Extract	6	21.34	2.54
G4 – Mildly Adulterated Extract	6	12.56	1.87
G5 – Highly Adulterated Extract	6	5.98	1.05

Table 4: ANOVA Summary

Source	SS	df	MS	F	Sig. (p-value)
Between Groups	5422.41	4	1355.60	172.84	0.000
Within Groups	196.34	25	7.85	—	—
Total	5618.75	29	—	—	—

- The F-value = 172.84 means that the difference between the groups is very strong.
- P-value = 0.000 (<0.05) is one of the indications that these differences are statistically significant.
- Therefore, pharmacological activity is highly variable based on the purity of samples detected by the FTIR.

This implies that adulteration directly affects the negative effect on the biological activity in animals.

➤ *Post Hoc Test (Tukey HSD)*

Tukey HSD is used in order to determine which particular groups are significantly different.

Table 5: Tukey HSD Group Comparisons

Group Comparison	Mean Difference	Sig.
Authentic vs Commercial	11.51	0.004
Authentic vs Mildly Adulterated	20.29	0.000
Authentic vs Highly Adulterated	26.87	0.000
Commercial vs Mildly Adulterated	8.78	0.018
Commercial vs Highly Adulterated	15.36	0.000
Mildly Adulterated vs Highly Adulterated	6.58	0.044

It was found that all the comparisons between the groups were significant by the Tukey HSD analysis and there was definite difference in pharmacological efficacy according to the levels of purity determined by the FTIR fingerprinting technique. The greatest therapeutic effect was observed with authentic extracts, moderate with commercial extracts, mildly adulterated samples showed a significant decrease and highly adulterated samples exhibited a minimum biological effect. This trend validates the fact that pharmacological performance is proportionate to the extent of adulteration, i.e. the FTIR spectral profiles that have been altered. Since the ANOVA values ($p = 0.000$) show a high level of variation between groups, and the Tukey HSD values show that there is a significant level of differences in the pair of samples, the null hypothesis (H_0) is rejected and the alternative hypothesis (H_a) accepted. In general, the results confirm that FTIR fingerprinting can accurately identify adulteration and strongly predicts decreased therapeutic efficacy in Wistar rats making it an effective predictive method of biological behavior of herbal raw materials.

4. DISCUSSION

The present research showed the usefulness of the combination of DNA barcoding, FTIR fingerprinting, and pharmacological analysis to verify the herbal raw materials and identify adulteration. The overall results emphasize the severe effect of adulteration on chemical

composition and efficacy of the therapeutic effect. The study gives a comprehensive insight into the effects of purity on the quality of herbal medicines in general by studying molecular, chemical, and biological parameters.

4.1. Interpretation of Results

The DNA barcoding outcome showed that genuine samples of *Gymnema sylvestre*, *Curcuma longa* and *Trigonella foenum-graecum* exhibited high value of sequence similarity (98% and above), which proved their botanical identity. Conversely, the percentages of similarity in adulterated samples were significantly lower suggesting the presence of foreign material or substitution. These results confirm the supposition that DNA barcoding is a very dependable instrument of species-based authentication of herbal products.

FTIR fingerprinting also identified authentic and adulterated samples by differences in their spectrals, with the differences showing clear chemical differences which could be attributed to adulterations. The clustering based on PCA was used to reinforce the differentiation, showing that the FTIR is capable of identifying fine changes in composition that other types of analysis might fail to detect.

These analytical observations were supported by pharmacological assessment. Rats administered with the real extracts had a significant decrease in blood glucose level than those treated with commercial extracts or adulterated extracts. Samples with the highest adulteration had the poorest therapeutic effect and the ANOVA outcome proved that the differences were statistically significant ($p < 0.001$). The Tukey test showed significant pairwise differences among all the groups and this showed that there was a good correlation between the purity detected by FTIR and the biological effect. The hypothesis that chemical adulteration has a deleterious effect on the pharmacological effects is confirmed by these findings.

4.2. Comparison with Existing Studies

The current study results are in line with earlier studies that showed that DNA barcoding and FTIR spectroscopy are reliable methods of authenticating herbs and identifying adulteration. DNA barcoding was found to identify species accurately whilst FTIR identified chemical adulterants according to previous studies. The decreasing pharmacological activity of the adulterated samples also concurs with the reports that impurities decrease the therapeutic activity.

Some of the significant studies to reinforce these observations are summarized in Table 6.

Table 6: Comparison with Existing Studies on Herbal Authentication

Study	Plant Material	Method	Key Findings
Noviana et al., (2022) ¹¹	Various herbal medicines	FTIR	Confirmed FTIR as reliable for quality control.

Osman et al., (2019)¹²	Herbs and spices	Analytical tools	Highlighted molecular and chemical methods for adulterant detection.
Seethapathy et al., (2018)¹³	Garcinia fruits	DNA barcoding, NMR	Successfully identified adulteration in supplements.
Urumarudappa et al., (2025)¹⁴	Plant extracts	Phytochemical authentication	Validated combined molecular and spectroscopic approaches.
Urumarudappa et al., (2016)¹⁵	Saraca asoca	DNA barcoding, NMR	Detected species adulteration in raw herbal trade.

This table affirms the assumption that DNA barcoding and FTIR is a good system to herbal authentication and quality assurance reliability.

4.3. Implications of findings

The application of DNA barcoding, FTIR fingerprinting, and pharmacological testing in a single approach will offer a framework of quality assurance in the herbal industry. The advantage of this combined method is that incorrect identification of the species as well as the presence of chemical adulterants and evaluation of their biological significance is detected. The findings emphasize the imminent necessity of regulatory bodies, manufacturers, and researchers to embrace multi-level authentication instruments in order to counter adulteration, enhance consumer protection as well as ensure therapeutic effectiveness of herbal products. These strategies can greatly enhance the pharmacovigilance practice and ensure international practices in manufacturing the herbal medicines.

4.4. Limitations of the Study

Although the study has strong outcomes, it is limited in some aspects. A limited number of three herbal species were chosen, which limits the applicability of the results to the larger herbal market. The animal experiment was offered in the course of a short period of 14 days which restricted the provision of long term pharmacological and toxicological impacts. Also, FTIR can also be used to detect moderate to heavy adulteration, but its sensitivity can be less in trace adulterants which may be detected using higher-order chromatographic techniques (such as HPLC or LC-MS).

4.5. Future Recommendations

Further studies and various medicinal species of plants should also be used to confirm the generalizability of the methods on herbal raw materials of different species. The assay would be enhanced with more sensitive methods of analysis (LC-MS, NMR, HPTLC) combined with FTIR to detect minor/complex adulterants even better. The animal model or clinical studies with longer duration are also advisable to determine long term safety and therapeutic results. Lastly, the centralized reference database that combines DNA barcodes and FTIR spectra of major

medicinal plants can make a significant contribution to the whole process of herbal authentication worldwide.

5. CONCLUSION

5.1. Summary of Key Findings

The research is able to show that DNA barcoding can well establish the species of the sampled herbal raw materials (*Gymnema sylvestre*, *Curcuma longa* and *Trigonella foenum-graecum*) with high precision of legitimate versus adulterated samples. FTIR fingerprinting was very effective in identifying chemical adulteration and offered classification with reference to the purity of samples. The pharmacological assessment on Wistar rats showed there was a great correlation between authenticity and therapeutic effect with the authentic extracts yielding the largest decreases in blood glucose and the adulterated extracts a gradual decrease in the biological activity. The significance of these observed values was verified by statistical tests (ANOVA and Tukey post hoc tests).

5.2. Significance of the Study

This study sheds light on the importance of adulteration in chemical structure as well as the pharmacological activity of herbal products. Through a combination of molecular, chemical and biological assessment, it offers an all-encompassing guideline to quality control, and it is a sure way in which manufacturers, researchers and regulatory agencies can protect consumer health and guarantee the efficacy of the therapy. The cumulative strategy underscores the importance of multi-level authentication devices within the herbal sector whereby international guidelines are encouraged when it comes to the quality control of herbal medicines.

5.3. Final Thoughts or Recommendations

Further studies ought to be extended to a greater range of medicinal plant species and apply more delicate analytical methods, e.g., LC-MS or HPLC, to identify the trace adulterants. To assess chronic effects and therapeutic outcomes, it is suggested to conduct long-term pharmacological and toxicological studies. It would be also effective to set up central databases of DNA barcodes and FTIR spectra of frequently used herbal species to enhance worldwide herbal authentication initiatives. On the whole, the paper highlights the role of combined authentication methods in ensuring the safety, effectiveness and dependability of herbal medicine.

REFERENCES

1. Anantha Narayana, D. B., & Johnson, S. T. (2019). DNA barcoding in authentication of herbal raw materials, extracts and dietary supplements: a perspective. *Plant Biotechnology Reports*, 13(3), 201-210.
2. Dev, S. A., Unnikrishnan, R., Jayaraj, R., Sujanal, P., & Anitha, V. (2021). Quantification of adulteration in traded ayurvedic raw drugs employing machine learning approaches with DNA barcode database. *3 Biotech*, 11(11), 463.
3. Gafner, S., Blumenthal, M., Foster, S., Cardellina, J. H., Khan, I. A., & Upton, R. (2023). Botanical ingredient forensics: Detection of attempts to deceive commonly used analytical

- methods for authenticating herbal dietary and food ingredients and supplements. *Journal of Natural Products*, 86(2), 460-472.
4. Kaur, R., Choudhary, T., Kaur, R., Garg, M., Chauhan, N. S., Jain, A., & Baldi, A. (2025). Adulteration in Herbal Medicines: A Comprehensive Review on Types, Detection, and Health Impacts. *Pharmacognosy Research*, 17(4).
 5. Kotsanopoulos, K. V., & Exadactylos, A. (2022). Methods and techniques for verifying authenticity and detecting adulteration. In *Authenticity of foods of plant origin* (pp. 32-82). CRC Press.
 6. Mohammed Abubakar, B., Mohd Salleh, F., Shamsir Omar, M. S., & Wagiran, A. (2017). DNA barcoding and chromatography fingerprints for the authentication of botanicals in herbal medicinal products. *Evidence-Based Complementary and Alternative Medicine*, 2017(1), 1352948.
 7. Naim, N., Ennahli, N., Hanine, H., Lahlali, R., Tahiri, A., Fauconnier, M. L., ... & Ennahli, S. (2022). ATR-FTIR spectroscopy combined with DNA barcoding and GC-MS to assess the quality and purity of saffron (*Crocus sativus* L.). *Vibrational Spectroscopy*, 123, 103446.
 8. Nazar, N., Saxena, A., Sebastian, A., Slater, A., Sundaresan, V., & Sgamma, T. (2025). Integrating DNA barcoding within an orthogonal approach for herbal product authentication: A narrative review. *Phytochemical Analysis*, 36(1), 7-29.
 9. Nehal, N., Choudhary, B., Nagpure, A., & Gupta, R. K. (2021). DNA barcoding: A modern age tool for detection of adulteration in food. *Critical reviews in biotechnology*, 41(5), 767-791.
 10. Nithaniyal, S., Vassou, S. L., Poovitha, S., Raju, B., & Parani, M. (2017). Identification of species adulteration in traded medicinal plant raw drugs using DNA barcoding. *Genome*, 60(2), 139-146.
 11. Noviana, E., Indrayanto, G., & Rohman, A. (2022). Advances in fingerprint analysis for standardization and quality control of herbal medicines. *Frontiers in Pharmacology*, 13, 853023.
 12. Osman, A. G., Raman, V., Haider, S., Ali, Z., Chittiboyina, A. G., & Khan, I. A. (2019). Overview of analytical tools for the identification of adulterants in commonly traded herbs and spices. *Journal of AOAC International*, 102(2), 376-385.
 13. Seethapathy, G. S., Tadesse, M., Urumarudappa, S. K. J., V. Gunaga, S., Vasudeva, R., Malterud, K. E., ... & Wangensteen, H. (2018). Authentication of *Garcinia* fruits and food supplements using DNA barcoding and NMR spectroscopy. *Scientific Reports*, 8(1), 10561.
 14. Urumarudappa, S. K. J., Bommuluri, V., Chaturvedi, S., Mittal, A. K., Zhang, Y., Chang, P., & Swanson, G. (2025). Authentication Methods for Phytochemicals (Botanicals) in Plant Extracts and Dietary Supplements. *Journal of Dietary Supplements*, 22(5), 680-721.
 15. Urumarudappa, S. K. J., Gogna, N., Newmaster, S. G., Venkatarangaiah, K., Subramanyam, R., Saroja, S. G., ... & Ramanan, U. S. (2016). DNA barcoding and NMR spectroscopy-based assessment of species adulteration in the raw herbal trade of *Saraca asoca* (Roxb.) Willd, an important medicinal plant. *International journal of legal medicine*, 130(6), 1457-1470.