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Mapping Iron enrichment: Unveiling Geographical Variation in *Palm jaggery*, *Finger millet*, and *Boerhavia diffusa* (*Punarnava) root*

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Abstract

Introduction: Iron deficiency is a predominant nutritional problem in India, with high prevalence rates in women and children. Palm jaggery (*Borassus flabellifer* L.), Finger millet (*Eleusine coracana*), and *Punarnava root* (*Boerhavia diffusa*) are traditional foods, which are essential sources of dietary iron and other micronutrients for the tribal and rural communities. Geographical, environmental, and processing factors, however, cause very large variations in the iron content as well as bioavailability of these foods. This research seeks to chart the geographic diversity in iron fortification and related phytochemical constituents of such indigenous foods with the help of High-Resolution Accurate Mass Spectrometry (HRMS). Methodology: Optimized *Finger millet*, *Punarnava root*, and *Palm jaggery* samples were collected from different Indian regions and were subjected to metabolomic profiling on the Orbitrap Eclipse Tribrid Mass Spectrometer (Thermo Fisher Scientific). Data processing was done using Compound Discoverer 3.3.2.31, with online databases integrated for comprehensive metabolite identification and quantification. Results: HRMS analysis revealed considerable geographical disparity in iron content along with the iron-modulating phytochemical profile in all samples. Siderophores, sulfur- and selenium-containing compounds, polyphenols, and organic acids were the major metabolites recognised, all of which were found to contribute to iron binding, absorption, and antioxidant activity. Particularly, Tamil Nadu *Palm jaggery* had high levels of diselane and selenophosphate, Dehradun *Finger millet* had catechin and ferulic acid in huge quantities. Uttarakhand *Punarnava root* had high levels of citric acid and glycitein. These findings highlight the complex interaction between geographical location and the nutrition content in local foodstuffs. Conclusion: This research offers the initial holistic mapping of iron enrichment and phytochemical diversity in *Palm jaggery*, *Finger millet*, and *Punarnava root* in India. The findings underscore region-based dietary advice and the revival of native food systems for the prevention of iron deficiency and the improvement of public health. Future studies must prioritize bioavailability studies and the effect of traditional processing on these findings to deliver the results in the form of effective nutritional intervention.

Key words: Iron, Finger millet, *Punarnava* root, Palm jaggery, HRMS, Phytochemical, Geographical Location.

Introduction

Iron deficiency is still one of the most prevalent nutritional disorders in India, with considerable public health consequences, especially for women and children (1). Although the Indian diet is very largely plant-based and dependent on food sources of non-heme iron, food iron content and availability are highly dependent and fluctuating, and determined by crop genetics and environmental factors like soil type, climate, and traditional farming practices (2). Traditional foods like cereals *Finger millet*, sweeteners *Palm jaggery*, and medicinal roots punarnava have an important part in the nutritional security of tribal and rural people (5). Not only are these foods high in integral micronutrients such as iron, calcium, and zinc but also provide the staple diet and form the basis of cultural practices in various Indian regions. *Finger millet* (*Eleusine coracana*) has a high iron content, with reported variation between landraces and growing locations, at times reaching over 12 mg/100 g (NEERUGANTI). *Palm jaggery*, made from unfermented sap of the palmyra palm, is prized for its mineral density and is traditionally suggested to control iron deficiency anemia (15; 19). *Punarnava root* (*Boerhavia diffusa*), which is commonly employed in Ayurveda, is another under utilised native food rich in iron and therapeutic value (20).

Though of nutritional importance, the iron content of these foods varies greatly according to their geographical origin. This variation is frequently due to variations in soil mineral content, local agro-climatic conditions, and traditional preparation practices. Nevertheless, standardised, region-specific data on the iron enrichment of these important foods are sparse and have been a discouraging barrier to making dietary advice and public health interventions more effective. Thus, the research aims to fill this void by using high-resolution mass spectrometry (HRMS) for comparing and contrasting the iron content and accompanying phytochemical profiles of these three traditional dishes, each derived from various Indian states. Through mapping regional iron enrichment differences, this research aims to provide evidence for the selection of improved nutrition food sources, facilitate indigenous food re-emergence and utilisation, and help mitigate iron deficiency in India.

Materials and Methods

Preparation of palm Jaggery, finger millet and Punarnava root

Palm jaggery was extracted from fresh sap of palmyra palm collected from different places. Sap was filtered and then warmed in a round-bottomed vessel at 120°C on an induction heater with constant stirring until the syrup became thick and dark brown in color. Heating was continued until the syrup reached a concentration of about 81° Brix total soluble solids. Once the desired concentration was attained, the blend was cooled to yield the final *Palm jaggery* product (8). Grains of *Finger millet* from Andhra Pradesh, Karnataka, and Dehradun were also sun-dried, washed, and ground into powder. *Punarnava root*s procured from Bihar, Madhya Pradesh, and Uttarakhand were also washed, dried in the sunlight, and powdered into an even powder for analysis.

All *Palm jaggery*, *Finger millet*, and *Punarnava root* samples were sourced from authenticated regional vendors: *Palm jaggery* from Andhra Pradesh, Tamil Nadu, and Kerala; *Finger millet* from Andhra Pradesh, Karnataka, and Dehradun; and *Punarnava root* from Bihar, Madhya Pradesh, and Uttarakhand. All samples were authenticated and accessioned in the Department of Rasa Shastra and Bhaisajya Kalpana (Ayurvedic Pharmacognosy) for traceability and botanical correctness. Authenticated samples were maintained in sealed containers at room temperature for future use.

Sample preparation for HRMS analysis

100 mg of each powder was weighed and placed in a sterile Eppendorf tube and 1.5 mL of the extraction solvent (methanol:water, 80:20 v/v) was added to this. The sample was homogenised in an Eppendorf ThermoMixer at 750 rpm for 30 minutes at 25°C and then centrifuged at 3500 rpm for 10 minutes at the same temperature. The supernatant was further filtered using a 0.22 µm PTFE syringe filter, and 4 µL of the filtered solution was utilised for future chromatographic separation (12).

Method used for HRMS analysis

HRMS analysis was carried out with the help of Mass Spectrometer (Thermo Fisher Scientific) with a DionexUltiMate 3000 RSUHPLC system as an accessory for phytochemical profiling (21). Chromatographic separation was performed on a Hypersil GOLD™ C18 RP-HPLC column (1.9 µm, 2.1 mm × 100 mm). The mobile phase was water with 0.1% formic acid and methanol with 0.1% formic acid, using a gradient program: 0–6 min, 5% MeOH; 6–10 min, 30% MeOH; 10–20 min, 50% MeOH; 20–25 min, 90% MeOH; 25–27 min, 90% MeOH; and 27–30 min, 5% MeOH, at 300 µL/min flow rate and column oven temperature of 40°C. The mass spectrometer was used in positive and negative ionisation modes, resolution 60,000, and m/z mass range 100–1000. Optimised conditions for RF Lens, AGC target, and collision energies were utilised, and data acquisition involved MS and ddMS2 scans (Singh et al., 2025).

Data processing and analysis

Raw data collected by the mass analyser were analysed using Compound Discoverer 3.3.2.31 (Thermo Fisher Scientific), utilising the Natural Product Unknown ID workflow. This method aligned retention times, identified and clustered unknown compounds, and predicted elemental compositions in all samples (11). There were blank samples to omit background signals. Compound identification was facilitated by mzCloud for spectral similarity searching, ChemSpider by exact mass or formula, and local database mass lists. For compounds with ddMS2 data, mzCloud was given preference for spectral matching, while ChemSpider and local databases were employed for mass-based identification. All experimentation and analysis were carried out at the Department of Rasa Shastra and Bhaishajya Kalpana, S.S.H, IMS, BHU, Central Discovery Centre (CDC)

Results

Overview of HRMS Analysis and metabolite detection

HRMS was used to perform detailed metabolic profiling of *Palm jaggery*, *Finger millet*, and *Punarnava root* samples obtained from India's varied geographical locations. The total ion chromatograms (TICs) for each sample offered a graphical overview of the total ion intensities that were detected on chromatographic separation, indicative of each food matrix's complexity and diversity of phytochemicals. The brief activity of each identified metabolite is mentioned in Table 1.

For every region and food type, typical ion chromatograms were obtained for the primary bioactive and iron-related compounds, allowing for accurate identification and quantification. The retention times (RT), computed molecular weights (MW), delta mass (ppm), and peak areas under the negative and positive ionisation modes for each metabolite are presented in the **Tables S1-S9** (Supporting information).

Palm jaggery: Regional metabolic profile

Palm jaggery samples from various Indian regions showed high variability in their phytochemical and bioactive content, as identified through high-resolution mass spectrometry. In the PAJN extract from Tamil Nadu, the total ion chromatogram showed a lush metabolite landscape with the principal iron-relevant sulfur- and selenium-containing compounds identified by HRMS analysis as diselane (RT 0.045 min, Area NEG: 1,385,197), selenophosphate (RT 0.643 min, Area NEG: 3,622,255), and histidinate (RT 4.083 min, Area NEG: 6,216,830). The sample also contained adenosine 5'-monophosphate (RT 0.397 min, Area POS: 1,560,692) and 5-mercaptotetrazole-1-acetic acid (RT 0.382 min, Area POS: 9,648,484), both sulfur-rich compounds that may support iron metabolism and absorption. *Palm jaggery* from Kerala (PJK) was characterised by its high content of organic acids known for enhancing iron bioavailability, specifically citric acid (RT 0.965 min, Area NEG: 2.41E+09) and azelaic acid (RT 13.896 min, Area NEG: 84,935,275), as well as polyphenols such as caffeic acid (RT 7.331 min, Area NEG: 24,892,008), ferulic acid (RT 10.147 min, Area POS: 67,241,493), apigenin (RT 12.871 min, Area POS: 15,618,703), taxifolin (RT 13.047 min, Area POS: 8,602,016), and diosmetin (RT 13.209 min, Area POS: 16,968,214), all known for antioxidant activity and iron-modulating potential. The presence of D-(-)-quinic acid (RT 0.926 min, Area NEG: 2.59E+08) and lariciresinol 4-O-glucoside (RT 12.912 min, Area NEG: 3,978,723) further supports the nutritional and functional richness of this sample. Concurrent *Palm jaggery* (PJA) from Andhra Pradesh showed a complex chromatographic profile with high levels of staphyloferrin B (RT 0.877 min, Area NEG: 64,938,917)—a strong siderophore enhancing iron bioavailability—and isoscutellarein 7-(6"-acetylallosyl-glucoside) (RT 13.699 min, Area POS: 1.1E+08), alongside various flavonoids and phenolic acids. Additionally, 2-mercaptobenzothiazole (RT 16.26 min, Area POS: 5,597,683), a sulfur-containing compound potentially supporting iron metabolism, was detected. Although isophthalic acid (RT 28.737 min, Area POS: 633,992) is present, it is less clearly linked to iron enrichment (Figures 1-3).

Table 1: Phytochemical components isolated by HRMS

S.No.	Food Source	Phyto-chemical Constituent	Reported Role in Iron Nutrition/Bioavailability
1	*Palm jaggery*	Diselane, Seleno-phosphate	Enhances iron absorption; sulfur and selenium compounds may support iron metabolism
		Bacillibactin C, Anguibactin	Siderophores with strong iron-chelating properties, improving iron bioavailability
		Cadmium Sulfide	Antimicrobial agent; directly improve food safety and iron stability
2	Finger millet	Catechin, Rutin, Ferulic acid	Polyphenols that can modulate iron absorption; some enhance, others may inhibit
		Bacillibactin C	Improves iron bioavailability
		Kojic acid, Azelaic acid	Known enhancers of non-heme iron uptake
3	*Punarnava root*	Glycitein, Genistein	Isoflavones that may act as iron chelators or enhancers, depending on context
		Phloroglucinol, Malic acid	Organic acids that can improve iron solubility and absorption
		Citric acid	Organic acid that improves iron solubility and absorption

Figure 1: Representative total ion chromatogram (TIC) of PJA *Palm jaggery* sample acquired using UHPLC-HRMS

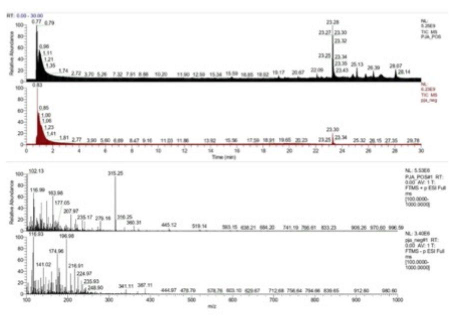


Figure 2: Representative TIC of PJK *Palm jaggery* sample acquired using UHPLC-HRMS

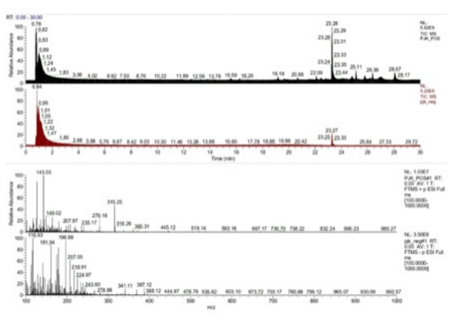
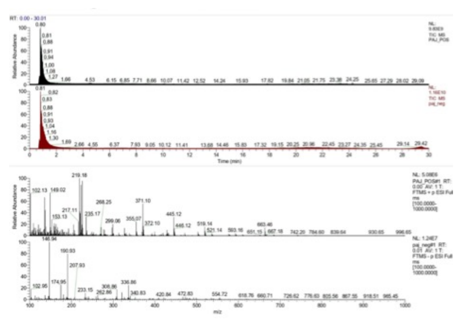


Figure 3: Representative TIC of PAJN sample acquired using UHPLC-HRMS



Finger Millet: Regional metabolic profile

Finger millet also exhibited significant regional diversity in metabolite composition, especially in compounds associated with antioxidant and iron-modulating activities. The Dehradun FMK sample showed elevated peaks for catechin (RT 1.203 min, Area POS: 9,760,000,000), ferulic acid (RT 10.293 min, Area POS: 97,069,085), rutin (RT 11.043 min, Area NEG: 7,679,455), quercetin (RT 11.294 min, Area POS: 13,384,122), and azelaic acid (RT 11.060 min, Area POS: 19,226,412). Interestingly, kojic acid (RT 0.947 min, Area POS: 33,880,001) and 3-hydroxyanthranilic acid (RT 0.984 min, Area POS: 63,746,376) were found, both of which are known to increase iron absorption. The detection of several flavonoids and organic acids in this sample highlighted the potential of this region's *Finger millet* as a promoter of health. In Andhra Pradesh, FMA *Finger millet* sample was characterised by high amounts of catechin (RT 1.222 min, Area POS: 9,520,000,000), phloretin (RT 13.633 min, Area POS: 16,647,389), naringenin (RT 9.299 min and 12.503 min, Area POS: 19,350,501 and 55,724,659), linoleoyl ethanolamide (RT 24.328 min, Area POS: 178,000,000), and azelaic acid (RT 11.022 min, Area POS: 21,813,254). The identification of luteolin (RT 14.582 min, Area NEG: 15,582,834), kaempferol (RT 16.556 min, Area POS: 3,337,880), and apigenin (RT 16.845 min, Area POS: 15,083,698) also corroborated the antioxidant and iron-modulating activities of this type of millet (Figures 4-6). Conversely, the RM sample from Karnataka was marked by a specific metabolic profile, which indicated high abundance of bacillibactin C (RT 10.795 min, Area NEG: 10,260,358), chelirubine (RT 11.068 min, Area NEG: 9,346,465), anguibactin (RT 13.892 min, Area NEG: 12,697,879), and elaiomycin F (RT 17.968 min, Area POS: 97,398,908). Identification of corrinoid (RT 17.92 min, Area NEG: 95,944,170) and histargin (RT 19.53 min, Area POS: 13,944,637) indicated abundance in iron-binding ligands and co-factors, whereas rare metabolites like pulcherrimin (RT 14.427 min, Area NEG: 10,270,400) and spinochalcone C (RT 19.573 min, Area NEG: 10,509,635) indicated region-specific abundance of bioactives. Collectively, these profiles indicate that both *Finger millet*s from these regions are an extensive source of bioactive flavonoids and organic acids, justifying its use in traditional diets to enhance iron status and general health.

Punarnava root: Regional metabolic profiles

Traditional medicine's staple, *Punarnava root*, contained a wide variety of phytochemicals in various Indian regions, most of which are associated with antioxidant and iron-binding activity. The Uttarakhand BDU sample contained elevated amounts of citric acid (RT 0.927 min, Area NEG: 8.28E+08), glycitein (RT 18.987 min, Area POS: 157,000,000), genistein (RT 10.242 min, Area NEG: 2,364,464), and phloroglucinol (RT 14.565 min, Area NEG: 11,002,535). The detection of coumestrol (Area POS: 53,468,099, RT 21.442 min) and hispidulin (Area NEG: 4,196,782, RT 11.32 min) reflected a high antioxidant and iron-binding activity in line with the therapeutic fame of *Punarnava root*. In Madhya Pradesh, the BDR sample had intense malic acid (RT 0.837 min, Area NEG: 2.03E+09), salicylic acid (RT 4.037 min, Area NEG: 1.06E+08), syringic acid (RT 5.102 min, Area NEG: 1.45E+07), xanthurenic acid (RT 6.218 min, Area POS: 1.25E+07), and catechin (RT 7.219 min, Area POS: 1.26E+08). Identification of isoferulic acid (RT 10.991 min, Area NEG: 7,752,071) and phloroglucinol (RT 14.565 min, Area NEG: 11,002,535) again supplemented the iron-modulating phytochemical matrix. The BDB sample from Bihar was characteristic for (R)-2-ethylmalic acid (RT 0.906 min, Area POS: 2.07E+08), citric acid (RT 0.919 min, Area NEG: 1.08E+09), catechin (RT 1.211 min, Area POS: 59,054,665), ferulic acid (RT 10.249 min, Area POS: 1.51E+08), and N-feruloyloctopamine (RT 10.995 min, Area NEG: 8,042,937) (Figures 7-9). Existence of classic and new iron-binding ligands, as well as several flavonoids, was proven.

Figure 4: Representative TIC of FMA sample acquired using UHPLC-HRMS

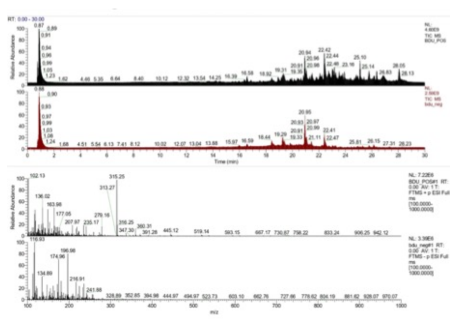


Figure 5: Representative TIC of FMK sample acquired using UHPLC-HRMS

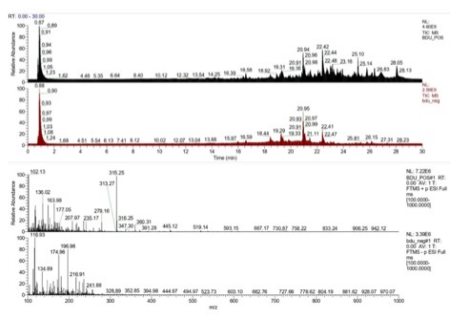


Figure 6: Representative TIC of RM *Palm jaggery* sample acquired using UHPLC-HRMS

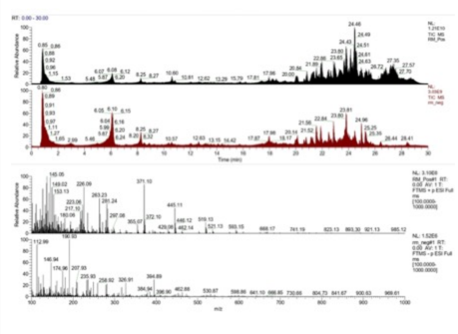


Figure 7: Representative TIC of BDB sample acquired using UHPLC-HRAMS

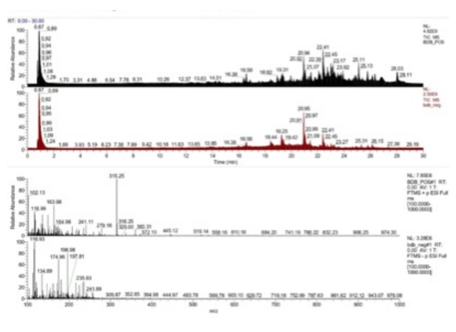


Figure 8: Representative TIC of BDR sample acquired using UHPLC-HRAMS

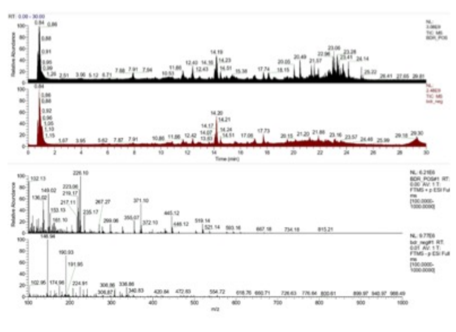
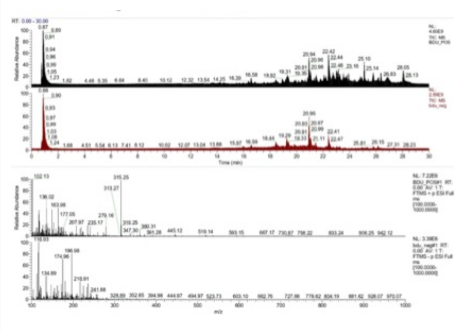


Figure 9: Representative TIC of BDU sample acquired using UHPLC-HRAMS



Overall, the metabolic profiling of *Palm jaggery*, *Finger millet*, and *Punarnava root* from different regions presents a rich and diverse landscape of bioactive compounds, with each region conferring distinctive nutritional and functional attributes to these conventional foods. The presence of actual RT and area values for predominant metabolites highlights the quantitative and qualitative significance of differences between regions and types of foods.

Comparative analysis

Comprehensive profiling of the HRMS of *Palm jaggery*, *Finger millet*, and *Punarnava root* samples from various Indian locations indicated large geographical variability in both iron enrichment and the phytochemical constitution linked with it. *Palm jaggery* samples from the Tamil Nadu (PAJN) was characterized by high abundance of sulfur- and selenium-containing compounds like diselane and selenophosphate, together with histidinate and adenosine 5'-monophosphate. These metabolites have been found to facilitate iron absorption and suggest that *Palm jaggery* of this region may possess improved nutritional qualities. PJA and PJK samples from Andhra Pradesh and Kerala were also enriched with flavonoids such as apigenin, taxifolin, and diosmetin, phenolic acids such as caffeic and ferulic acid, and organic acids such as citric and azelaic acid. These are widely reported to possess dual activities of iron absorption promoters as well as antioxidants, thereby validating the food functional status of these regions for *Palm jaggery*.

Dehradun *Finger millet* (FMK) and Andhra Pradesh *Finger millet* (FMA) too showed regional variation. The FMK sample stood out for containing extremely high levels of catechin, ferulic acid, rutin, and quercetin, along with iron absorption promoters such as kojic acid and azelaic acid. The FMA sample, although just as rich in catechin, contained a wider range of flavonoids such as phloretin, naringenin, and linoleoyl ethanolamide. Both of the samples were rich in polyphenols and organic acids, which highlight their worth as iron and antioxidant-rich foods. *Finger millet* Karnataka (RM) exhibited high production of siderophores like bacillibactin C and anguibactin, and new bioactive metabolites like chelirubine and elaiomycin F. These iron-chelators suggest a high value for iron bioavailability enrichment of this local food.

Both intra-species and inter-regional variation in each of the *Punarnava root* samples from Uttarakhand (BDU), Madhya Pradesh (BDR), and Bihar (BDB) were highly significant. The BDU sample had very high levels of citric acid, glycitein, genistein, and phloroglucinol that are known to increase iron bioavailability. The BDR sample contained high levels of malic acid, salicylic acid, syringic acid, and catechin with supporting contributions provided by isoferulic acid and phloroglucinol. The BDB sample alone was distinctive in its blend of (R)-2-ethylmalic acid, citric acid, catechin, ferulic acid, and it thus carries a particular regional phytochemical signature.

Throughout all samples, the diversity and abundance of iron-binding and iron-enriching metabolites were the greatest in areas with iron-rich soil and traditional processing practices.

Siderophores and sulfur/selenium compounds were especially stand-out in some of the *Palm jaggery* samples, whereas polyphenols and organic acids were the major contributors in *Finger millet* and *Punarnava root* samples. The high content of polyphenols and flavonoids in all samples indicates their potential not just in micronutrient fortification but also in imparting all-around antioxidant and health effects.

In general, this comparative study clearly shows that phytochemical composition and iron enrichment in *Palm jaggery*, *Finger millet*, and *Punarnava root* are geographically highly variable. Every region and type of food provides a distinct nutritional and bioactive fingerprint, and thus the significance of indigenous food systems and the requirement of region-specific diets to curb iron deficiency effectively in India.

Discussion

This research offers exhaustive mapping of iron enrichment and linked phytochemical profiles in *Finger millet*, *Palm jaggery*, and *Punarnava root* from various Indian sites by HRMS. The findings reveal extensive geographical variability in the abundance and diversity of iron-binding, iron-enriching, and antioxidant metabolites in all three foods. The variety highlights the contribution of indigenous agro-ecological environments, traditional processing, and crop genotypes in determining the nutritional quality of traditional foods. Of interest here is the occurrence of sulfur- and selenium-based molecules such as diselane and selenophosphate in Tamil Nadu *Palm jaggery*. These molecules are also found to enhance iron uptake and may be the reason for the improved nutritional content of *Palm jaggery* here (13). The presence of siderophores like bacillibactin C and anguibactin in Maharashtra *Palm jaggery* suggests a mechanism separate from that described earlier because siderophores are well known to exhibit strong iron-chelating affinity (6).

Dehradun *Finger millet* had high contents of catechin, rutin, and ferulic acid, all earlier reported to have been involved in iron absorption and exhibit antioxidant activity (3). The presence of kojic acid and azelaic acid, the latter also acting as stimulants of non-heme iron absorption, also bears witness to *Finger millet*'s nutritional value as a functional food. These findings corroborate the previous findings that *Finger millet* iron bioavailability and content are strongly genotype- and region-specific (18). The phytochemical content of *Punarnava root* samples was diverse in intra-species and inter-regional terms. The abundance of the samples in isoflavone, organic acid, and phenolics suggests the presence of a synergistic effect in promoting iron bioavailability and antioxidant activity (9). Such phytochemical diversity can also augment the drug-like potential of *Punarnava root*, which has been greatly valued in Ayurveda down the centuries for its medicinal worth.

The comparative analysis identified that the highest iron-modulating abundance and diversity of metabolites were found in samples from sites with naturally iron-rich soils and traditional processing. This verifies the hypothesis that environmental and cultural differences play a crucial role in determining the nutritional profile of traditional Indian food (4).

Furthermore, the fact that the evidence for high content of flavonoids and polyphenols in all the samples also certifies the role played by such foods in micronutrient supplementation along with other benefits towards general well-being such as antioxidants as well as anti-inflammatory effect (14). These findings also indicate local nutrition guidelines and the reconstruction of indigenous food systems in a bid to properly deal with iron deficiency as well as improve public health status in India.

Conclusion

The current study offers a high-resolution map of iron enrichment and phytochemical diversity of *Palm jaggery*, *Finger millet*, and *Punarnava root* of various geographical origins of India as analysed by high-resolution mass spectrometry showing vast geographic variation in iron concentration and accompanying bioactive molecules. The occurrence of significant iron-binding and iron-enriching metabolites such as siderophores, sulfur- and selenium-containing compounds, polyphenols, and organic acids is evidence of the nutritional and medicinal significance of such autochthonous foods and underscores the contributions played by autochthonous factors such as soil chemistry and native food preparation techniques. These results applaud the appropriateness of regional dietary advice and the revival of indigenous food systems for nutritional security and the prevention of iron deficiency anemia. Direct measurement of bioavailable iron, in vivo absorption tests, and impacts of representative cooking on nutrient retention are areas of future research to take these results to the field of practical dietary recommendations. Widening the scope to more geographic areas and less explored food items will add greater insight into India's nutritional spectrum. Generally, the work has significant implications for food policy and public health nutrition, giving evidence to support evidence-informed targeted interventions that maintain cultural dietary habits but fix micronutrient deficiencies sustainably and effectively.

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