

LC–MS/MS-based metabolomic profiling of *Syzygium cumini* (L.) Skeels: Identifying key phytochemicals driving α -amylase inhibition and antioxidant activity

Ajay Kumar

Indian Agricultural Research Institute

Kanhaiya Singh

ksinghiari@yahoo.co.in

Indian Agricultural Research Institute

Jai Prakash

Indian Agricultural Research Institute

Amit Kumar Goswami

Indian Agricultural Research Institute

Vishaw Bandhu Patel

Indian Council of Agricultural Research

Amit Kumar Singh

National Bureau of Plant Genetic Resources

Yamini Yadav

Indian Agricultural Research Institute

Aditya Dnyaneshwar Ingole

Indian Agricultural Research Institute

Bhupendra Sagore

Indian Agricultural Research Institute

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Abstract

Indian blackberry (*Syzygium cumini* (L.) Skeels) possesses immense pharmacological potential, yet unlocking the mechanisms behind these health benefits requires a precise elucidation of the fruit's complex metabolic architecture. This study provides a comprehensive LC–MS/MS-based profiling of bioactive compounds and sugars in three contrasting genotypes (PCJ-9, 15, 17) across pulp and seed tissues. We quantified 49 metabolites, identifying distinct tissue-specific fingerprints via OPLS-DA ($Q^2 = 0.756\text{--}0.988$). Seeds were enriched with phenolics (rutin, gallic acid), while pulp dominated in anthocyanins and soluble sugars. Correlation analysis revealed that total flavonoid content (TFC), specifically catechin and protocatechuic acid, rather than total phenolics, is the primary driver of antioxidant and α -amylase inhibitory activities ($r = 0.95\text{--}0.97$). The small-seeded PCJ-15 genotype emerged as the most potent therapeutic candidate. KEGG pathway analysis revealed a metabolic "hub" in flavonoid biosynthesis and identified a significant inverse correlation between primary sugar accumulation and secondary metabolite synthesis ($r < -0.70$). This suggests that *S. cumini* operates as a naturally optimized delivery system, providing high-density phytochemicals with minimal glycemic impact. These findings establish a mechanistic foundation for the clinical application of jamun-derived bioactives in managing metabolic disorders, aligning with the therapeutic goals of functional food development.

Introduction

Syzygium cumini L. (family: Myrtaceae), popularly known as jamun, Indian blackberry, jambul, jambolana, danson plum, or black plum, is an underutilised tropical fruit tree with high horticultural and nutraceutical potential. Native to the Indian subcontinent, it is extensively grown throughout South Asia, including India, Myanmar, Nepal, Sri Lanka, Bangladesh, and Pakistan, and has been successfully domesticated in many regions of Asia, America, and Africa^{1–2}. Because of its flexibility, jamun flourishes even in marshes and marginal soils where other fruit trees are difficult to grow commercially. The fruit, with its solitary seed and deep purple flesh, is eaten fresh or made into juices, jellies, jams, vinegars, wines, puddings, ice creams, and fruit strips^{4–5}. *S. cumini* has a long history in Ayurvedic, Unani, and Chinese medicine, where various plant parts are used to treat hyperglycemia, diarrhoea, glycosuria, asthma, bronchitis, ulcers, and other conditions⁵. Pharmacological investigations have shown its anti-anaemic, antioxidant, antibacterial, antiallergic, hypolipidemic, hepatoprotective, antipyretic, and, most importantly, antidiabetic properties^{6–7}. These therapeutic benefits are primarily attributed to a diverse array of bioactive chemicals, including phenolic acids, flavonoids, and anthocyanins, which modulate metabolic pathways through free radical scavenging and metal-chelating activities^{8–10}. Furthermore, the sugar content influences sweetness, glycaemic index, and total nutritional value. Jamun pulp's low glycaemic index and specific sugar profile make it acceptable for diabetics, while minor sugars and seed-derived bioactives help regulate glucose metabolism and insulin sensitivity¹¹.

The genus *Syzygium* is economically important due to its extraordinary bioactivity and potential use in food and medicine¹². Despite its medicinal potential, *S. cumini* seeds and pulp have historically lacked a high-resolution metabolite profile, particularly regarding the synergy between polyphenols and the sugar matrix. The sugar profile has frequently been overlooked or evaluated only as total soluble sugars, without the structural characterization necessary to understand its influence on the glycemic index and glucose metabolism.

Understanding the mechanisms underlying these health effects requires a precise understanding of the fruit's metabolic architecture. Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) provides the high-resolution separation and accurate structural identification required to decipher these complex metabolomic landscapes and their potential clinical relevance^{13–14}. Additionally, advanced analytical platforms such as LC–MS/MS

combined with multivariate statistical approaches, including PLS-DA and OPLS-DA, enable comprehensive interpretation of complex metabolomic datasets and facilitate the identification of key discriminatory metabolites^{15–16}.

Keeping in view the pharmacological potential of *Syzygium cumini*, the present study was conducted to establish a high-resolution metabolomic landscape of its pulp and seed tissues using LC–MS/MS. The primary objectives were to characterize the distribution of phenolic acids, flavonoids, anthocyanins, and sugars across three distinct genotypes and utilize OPLS-DA to identify the key discriminant metabolites. Furthermore, the study aimed to evaluate the antioxidant and alpha-amylase inhibitory potential of these tissues, integrating the results with KEGG pathway analysis to elucidate the biosynthetic origins of these health-promoting phytochemicals. By bridging metabolic diversity with functional bioactivity, this work seeks to provide a scientific basis for the use of *S. cumini* in targeted nutraceutical interventions for metabolic health.

Result and discussion

Phenolic acids profiling of pulp

The LC-MS/MS profiling of *S. cumini* pulp reveals a significant genotype-specific accumulation of phenolic acids, suggesting that genetic factors are primary determinants of the fruit's secondary metabolite profile (Table 1; Fig. 1). The total phenolic acid content was found to be 714.16 µg/g FW in PCJ-17 and 1030.67 µg/g FW in PCJ-15, with PCJ-9 showing an intermediate level of 831.25 µg/g FW. Among the phenolics, benzoic acid was the principal one, having the highest accumulation in PCJ-15 (660.14 µg/g FW), and then follow PCJ-9 (483.07 µg/g FW), and lastly, PCJ-17 (451.67 µg/g FW). Gallic acid was second in terms of abundance, with its maximum in PCJ-15 (313.58 µg/g FW) and its minimum in PCJ-17 (223.68 µg/g FW). The highest level of vanillic acid was found in PCJ-17, whereas ellagic and chlorogenic acids were mainly present in PCJ-15, indicating a genetic influence on metabolic distribution. Fruits such as blueberries and raspberries are likewise abundant in hydroxybenzoic and hydroxycinnamic acids, the metabolites that have received widespread recognition for their antioxidant and nutraceutical potential¹⁷. Active cellular metabolism during fruit maturation in jamun pulp likely promotes the biosynthesis of phenolic acids via the shikimate pathway, thereby enhancing both fruit quality and its defensive capacity¹⁸. The study at present found moderate amounts of p-coumaric, o-coumaric, cinnamic, syringic, and gentisic acids, besides trace quantities of caffeic, ferulic, and sinapic acids. The highest phenolic diversity was exhibited by PCJ-15, while PCJ-17 had a narrower profile characterised by benzoic and vanillic acids.

Phenolic acids profiling of seed

The seeds contained much higher concentrations of phenolic acids than the pulp and significantly varied from 826.52 µg/g FW in PCJ-15 to 1618.00 µg/g FW in PCJ-9. Gallic acid was the predominant seed phenolic, being highest in PCJ-9 (919.73 µg/g FW) and PCJ-17 (830.05 µg/g FW), with a relatively low level recorded in PCJ-15 (492.44 µg/g FW). Ellagic acid was highest in PCJ-9, at 396.02 µg/g FW, but was recorded at minimal levels in PCJ-17. Also, vanillic acid varied only between 8.12–12.63 µg/g FW among genotypes, indicating very limited variation among the genotype means. PCJ-15 uniquely accumulated sinapic (22.67 µg/g FW) and protocatechuic acid (0.59 µg/g FW), while other phenolics-p-coumaric, o-coumaric, cinnamic, syringic, and ferulic acids-showed moderate variation. Minor acids such as caffeic, salicylic, and chlorogenic acids were detected only in trace amounts (Table 1; Fig. 1). Seeds usually store structural and defensive phenolics for long-time viability, while pulp phenolics mainly provide antimicrobial and oxidative protection during ripening¹⁸. The very high gallic acid content in PCJ-9 and PCJ-17 indicates high radical-scavenging potential, in agreement with its well-documented antioxidant properties¹⁹. Similarly, the very high ellagic

acid in PCJ-9 agrees with reports that identified jamun seed ellagitannins as key contributors to antioxidant and antiproliferative activity²⁰⁻²¹. Comparative analysis confirmed that seeds consistently accumulated higher phenolic acid levels than pulp in all genotypes (Fig. 2E). This is metabolically important since tissue-specific variation implies that hydroxybenzoic acids influence sensory quality, while hydroxycinnamic acids enhance oxidative stability and bioactivity²². PCJ-15 exhibited the highest total phenolic content in both pulp and seed, although PCJ-9 dominated in gallic and ellagic acids in seeds, indicating genotype-dependent biosynthesis and partitioning patterns, in agreement with earlier reports on underutilized fruits, including jamun²³.

Flavonoid profiling of pulp

LC-MS/MS profiling yielded genotype-specific flavonoid fingerprints in pulp of jamun and, in total, 15 flavonoids were detected as depicted in Table 2 & Fig. 2. The total flavonoids ranged from 3088.7 ng g⁻¹ FW in PCJ-17 to 35,298.3 ng g⁻¹ FW in PCJ-15. PCJ-15 showed very high values of myricetin (27,111.7 ng g⁻¹ FW), catechin (6,046.7 ng g⁻¹ FW), and hesperetin (696.3 ng g⁻¹ FW) as compared with other genotypes. On the other hand, PCJ-17 accumulated more epigallocatechin (766.1 ng g⁻¹ FW) and moderately higher quercetin (530.2 ng g⁻¹ FW), while PCJ-9 had intermediate values for most flavonoids but significantly higher rutin (831.4 ng g⁻¹ FW) accumulation. These observations are in agreement with previous studies reporting the rich flavonoid diversity of *Syzygium cumini* pulp^{22,24}. Pulp tissue is made up of metabolically active parenchyma, which supports the synthesis of free catechins and flavonols like hesperetin that contribute to antioxidant defence, pigmentation, and sensory attributes²⁵. Myricetin is documented as a powerful antioxidant with metal-chelating ability and has been widely reported in jamun and berries²⁶⁻²⁷. Interestingly, the exceptionally high catechin and hesperetin levels in PCJ-15 significantly enhance its nutraceutical potential, owing to their documented cardioprotective and anti-inflammatory functions²⁸⁻²⁹. The high amount of rutin in PCJ-9 manifests the vasoprotective and anti-inflammatory potential⁸.

Flavonoid profiling of the seed

Seed flavonoid profiles were markedly different across the genotypes, with PCJ-15 having the highest total flavonoids (33,306.11 ng g⁻¹ FW), followed by PCJ-9 (23,322.66 ng g⁻¹ FW) and PCJ-17 (10,197.85 ng g⁻¹ FW) (Table 2; Fig. 2). Rutin was the dominant seed flavonoid, peaking in PCJ-15 (20,331.2 ng g⁻¹ FW), followed by PCJ-9 (10,369.1 ng g⁻¹ FW) and lowest in PCJ-17 (3,230.4 ng g⁻¹ FW). In addition, PCJ-9 displayed elevated naringenin (3,232.3 ng g⁻¹ FW) and epigallocatechin (5,720.1 ng g⁻¹ FW); PCJ-17 was accumulated with more epicatechin (3,029.9 ng g⁻¹ FW), while PCJ-15 showed the highest levels of catechin, quercetin, and hesperetin. The minor flavonoids presented genotype-specific distribution, including apigenin in PCJ-9, luteolin in PCJ-15, and eriodictyol in PCJ-17. Seeds manifested consistently higher flavonoid values than pulp, with rutin being the most prevalent, which is in accordance with previous reports³⁰⁻³¹. Rutin plays an important role in glucose regulation, antihypertensive defense, antioxidant defense, and α -glucosidase inhibition, which justifies the traditional use of jamun seeds against diabetes^{1,32}. Similarly, quercetin present in seeds exerts effective antioxidant and antidiabetic potentials³³. Comparative analysis thus established that seeds accumulated higher concentrations of flavonoids compared to pulp (Fig. 2e). This finding corroborates previous literature that evidenced greater phenolic and flavonoid accumulation in the seeds of jamun than in its pulp^{32,34}. Myricetin, quercetin, catechin, and rutin are established antioxidants⁸. Hence, their presence suggests that the studied genotypes represent rich repositories of natural bioactives. Genotype and tissue-dependent flavonoid composition, coupled with antioxidant potential variation, has similarly been recorded for grapes³⁵, blueberries³⁶, and pomegranate³⁷. These studies thus authenticate the findings of the present investigation.

Anthocyanin profiling of pulp

Anthocyanins in *Syzygium cumini* are mainly concentrated in the fruit pulp rather than in seeds, and the results presented here showed significant genotype-specific variation in pulp anthocyanin composition (Table 3; Fig. 3). The dominating anthocyanins of PCJ-15 were cyanidin (1076.03 µg/g FW) and delphinidin (2601.55 µg/g FW), thereby making a total of 3812.77 µg/g FW, the highest among genotypes. PCJ-9 had an intermediate concentration of cyanidin (296.85 µg/g FW) and delphinidin (363.39 µg/g FW) with a total of 829.36 µg/g FW, while PCJ-17 possessed an extremely low concentration of cyanidin (6.27 µg/g FW) and delphinidin (58.31 µg/g FW), hence totalling to 76.92 µg/g FW. Malvidin concentration was the highest in PCJ-9 (168.86 µg/g FW), while pelargonidin manifested marginal variation, ranging from 0.16 µg/g FW in PCJ-15 to 0.32 µg/g FW in PCJ-17. Anthocyanins are water-soluble flavonoid pigments that provide purple, blue, and red colouration; they participate in fruit defence and human health due to their strong antioxidant characteristics³⁸⁻³⁹. Observed variation with genotype- and tissue-specific anthocyanin composition matched earlier reports in jamun and other purple fruits²⁴⁻²⁷. Cyanidin and delphinidin, dominant in PCJ-15, are widely reported for potent radical scavenging, cardioprotective, and anti-inflammatory properties³⁹⁻⁴⁰. Malvidin accumulated in PCJ-9 pulp has been associated with anticancer and neuroprotective properties in many fruits⁴¹. Very low pelargonidin content indicated its minor role in jamun pigmentation, similar to that observed in blueberries and grapes⁴².

Anthocyanin profiling of the seed

Anthocyanin composition also varied significantly in seeds among the genotypes, although the concentration was much lower compared with the pulp (Table 3; Fig. 3). Delphinidin was the major seed anthocyanin that accumulated maximally in PCJ-9 (387.10 µg/g FW), followed by PCJ-15 (333.43 µg/g FW) and PCJ-17 (292.52 µg/g FW). Cyanidin was present in relatively low concentration, although its highest content was recorded in PCJ-9 (28.36 µg/g FW). Pelargonidin and malvidin occurred as minor anthocyanins, but maintained consistently higher concentration in PCJ-9 over other genotypes. Seeds of PCJ-9 showed the highest total anthocyanin content of 426.37 µg/g FW, followed by PCJ-15 (336.70 µg/g FW) and PCJ-17 (296.49 µg/g FW). The lower anthocyanin levels of the seeds corroborate jamun studies that state that seeds mainly accumulate non-pigmented phenolics, such as catechins, rather than colored anthocyanins. This is further reflected in similar behavior reported for pomegranate, where seed tissues are primarily composed of proanthocyanidins and catechin derivatives rather than pigment-rich anthocyanins³⁷. Comparative analysis indicated that the pulp of all the studied genotypes had significantly higher anthocyanin content than seeds. Nearly nine-fold higher anthocyanins accumulated in PCJ-15 pulp compared with its seeds, whereas moderate pulp-to-seed variations were observed for PCJ-9 and low contents in both tissues for PCJ-17. These observations are supported by earlier reports where jamun pulp was found to contain high levels of cyanidin-3-glucoside and delphinidin derivatives, while seeds primarily store non-pigmented phenolics for defense against stresses²¹⁻³⁴. Such tissue-specific compartmentalization is well documented in various pigmented fruits, where pulp pigmentation offers attraction, antioxidation, and stress tolerance activities, while the seeds predominantly focus on long-term defense metabolism³⁸⁻³⁹. Thus, this genotype × tissue interaction is useful for functional food design and bioactive enrichment through breeding programs^{24,27}.

Sugar profiling of pulp

LC–MS/MS analysis showed clear genotype-dependent variations in the sugars profile of jamun pulp (Table 4; Fig. 4). Jamun pulp sugars were dominated by glucose and fructose, which are the major sweetening sugars in ripe fruits, whereas sucrose either was not detected or was below the detectable limit⁴³. Among the monosaccharides, ribose ranged between 41.22 µg/g FW in PCJ-15 and 62.55 µg/g FW in PCJ-17, with PCJ-9 showing an intermediate level of 46.26 µg/g FW. Arabinose and xylose were present in lower levels compared to ribose, although arabinose was at its

highest (3.93 µg/g FW) and xylose was at its lowest in PCJ-15 (0.83 µg/g FW). These results are consistent with the occurrence of arabinose, xylose, and mannose in jamun fruit pulp⁴⁴. Significant differences were noted in rhamnose and fucose, where PCJ-9 showed the highest values for both sugars at 23.57 µg/g FW and 10.85 µg/g FW, respectively, while PCJ-17 showed the least concentration. Among the reducing sugars, fructose was the predominant sugar constituent: 73,960.67 µg/g FW (PCJ-9), 53,278.19 µg/g FW (PCJ-17), and 19,567.64 µg/g FW (PCJ-15). Glucose and mannose also showed remarkable variation; PCJ-15 had the highest mannose concentration (24,372.95 µg/g FW), while PCJ-9 accumulated the highest glucose (15,465.50 µg/g FW). Sugar alcohols, which include inositol and sorbitol, were detected in small quantities, with the highest amount found in PCJ-9 and PCJ-15, respectively. The disaccharides remained low within genotypes and are highest for sucrose in PCJ-9 (45.80 µg/g FW) and lowest in PCJ-15 (8.94 µg/g FW). Total sugar pulp varied widely and was highest in PCJ-9 (92,906.81 µg/g FW), followed by PCJ-17 (71,456.69 µg/g FW) and finally PCJ-15 (57,362.83 µg/g FW). These are within the reported range: fructose ~6.2 g/100 g and glucose ~5.0 g/100 g⁴⁵. Total soluble sugars ranged from 0.65 to 1.76 g/100 g according to the cultivar type⁴⁶.

Sugars profiling of the seed

LC–MS/MS analysis of jamun seeds indicated different patterns of sugar accumulation in the three genotypes studied (Table 4; Fig. 4). Among the monosaccharides, ribose varied from 5.54 µg/g FW (PCJ-17) to 8.06 µg/g FW (PCJ-9), while arabinose and xylose were present in relatively lower concentrations, with maximum xylose content in PCJ-15. The rhamnose content was highest in PCJ-9 (8.84 µg/g FW) and lowest in PCJ-15 (2.58 µg/g FW). Fucose also showed high variability, with a maximum value in PCJ-15 (6.35 µg/g FW) and a minimum in PCJ-17 (3.75 µg/g FW). Among the reducing sugars, fructose predominated in seeds and was highest in PCJ-9 (22,841.97 µg/g FW), followed by PCJ-15 (20,948.60 µg/g FW) and PCJ-17 (16,240.59 µg/g FW). Glucose and mannose were also highest in PCJ-9 at 4,958.21 µg/g FW and 2,818.02 µg/g FW, respectively. In support of these findings, glucose is the predominant seed sugar, while pulp is rich in glucose and fructose, contributing to sweetness⁴⁴. Sugar alcohols were present in trace quantities only. Of the disaccharides, sucrose was most abundant in PCJ-17 (117.53 µg/g FW) and lowest in PCJ-9 (17.97 µg/g FW). As expected, sorbitol is usually present in pulp in very small quantity, while arabinose is present as a trace component even when detailed profiling of sugars has been done (GC–MS)⁴³. The comparative assessment of genotypes clearly indicated that the total sugar content was greater in pulp than in seed in all genotypes. The maximum difference was found in PCJ-9 (92,906.81 µg/g FW in pulp vs 31,839.16 µg/g FW in seed). Pulp was mainly rich in fructose, which is the main sweetener affecting taste and palatability and the glycemic response, while seeds accumulated relatively higher glucose and mannose, associated with storage and metabolic stability. The distribution of sucrose in different tissues provides additional insight into the relationships between genotype and tissue in sugar metabolism. Understanding the sugar content of jamun is crucial for evaluating its sweetness, calorie contribution, and glycaemic response. It is especially noteworthy that jamun pulp has a favourable sugar profile and a low glycaemic index, making it advantageous for diabetic diets. Minor sugars and bioactives from seeds also support anti-diabetic action via insulin sensitization and inhibition of carbohydrate- hydrolyzing enzymes.

OPLS–DA analysis of metabolite profiles

OPLS–DA score plots revealing tissue-specific metabolite separation

The OPLS-DA score plots showed clear metabolic separation between pulp and seed samples, which indicated a strong tissue-specific metabolite differentiation. Component 1 represented the major variation that explained the discrimination, while Component 2 represented orthogonal variation unrelated to class separation. For phenolic acids, Component 1 explained 27.7% of the total variation. In comparison, Component 2 explained 24.6% (Fig. 1d). In

flavonoids, Component 1 contributed to 27.5%, while Component 2 captured 21.2% of the orthogonal variance (Fig. 2d). For anthocyanins, Component 1 explained 30.5%. In comparison, Component 2 explained 39.9%, reflecting very strong separation (Fig. 3d). In sugar metabolites, Component 1 contributed a comparatively larger proportion, 62.1%. In comparison, Component 2 accounted for 13.6%, reflecting strong discriminatory potential for primary metabolites (Fig. 4d). The pronounced metabolic differentiations between pulp and seed tissues detected in the non-overlapping grouping patterns of score plots confirmed the effectiveness of OPLS-DA in distinguishing pulp and seed of jamun based on phenolic acids, flavonoids, anthocyanins, and sugars. These metabolite groups represent major phytochemical classes associated with bioactive properties in fruits, highlighting the importance of tissue-specific metabolite accumulation in shaping the functional properties of *Syzygium cumini*. OPLS-DA has been widely applied in fruit metabolomics to reveal biochemical variation across tissues, genotypes, and developmental stages. Similar tissue-dependent metabolic discrimination has been reported in cashew apple⁴⁷, pomegranate tissues⁴⁸, and related metabolomics studies⁴⁹, supporting the reliability of OPLS-DA for tissue-targeted phytochemical profiling.

OPLS-DA model validation and predictive performance

OPLS-DA models constructed for phenolic acids, flavonoids, anthocyanins, and sugars showed a very good fit with high predictive ability and statistically significant class discrimination. For phenolic acids, the model gave $R^2X = 0.783$, $R^2Y = 0.999$, $Q^2 = 0.756$ (Fig. 1c). For flavonoids, the values were $R^2X = 0.778$, $R^2Y = 1.000$ and $Q^2 = 0.988$ (Fig. 2c), and for anthocyanins, the values were $R^2X = 0.778$, $R^2Y = 1.000$, $Q^2 = 0.988$ (Fig. 3c). The model for sugars gave $R^2X = 0.757$, $R^2Y = 0.984$ and $Q^2 = 0.968$ (Fig. 4c). Permutation testing confirmed the statistical significance of all models ($p < 0.005$, 0/200 permutations), indicating robust class separation and strong model reliability. The high R^2 values indicate excellent goodness of fit, while the high Q^2 values demonstrate strong predictive performance without evidence of overfitting. Comparable OPLS-DA model performances have been reported in fruit metabolomics studies, including cashew apple ($R^2X = 0.982$, $R^2Y = 0.979$, $Q^2 = 0.937$)⁵⁰ and muskmelon ($R^2X = 0.598$, $R^2Y = 0.972$, $Q^2 = 0.881$)⁵¹, supporting the reliability of the present models.

VIP score analysis for identification of key discriminant metabolites

The OPLS-DA-based VIP score analysis identified the key metabolites contributing to sample discrimination between pulp and seed. In the case of phenolic acids, benzoic (VIP = 1.78), gallic (1.72), chlorogenic (1.26), o-coumaric (1.26), p-coumaric (1.22), sinapic (1.01), and syringic acid (1.04) were the most influential contributors (Fig. 1d). For flavonoids, naringenin (VIP = 1.88), rutin (1.77), fisetin (1.51), epicatechin (1.45), and umbelliferone (1.08) played dominant roles, whereas epigallocatechin, eriodictyol, and myricetin showed moderate influence (VIP = 0.70–0.87) (Fig. 2d). In the case of anthocyanins, malvidin (VIP = 1.50) and cyanidin (VIP = 1.20) were the major discriminants (Fig. 3d). For sugars, arabinose, glucose, inositol, ribose, sorbitol, trehalose, and xylose (VIP > 1) were the most important classifiers, while fructose, fucose, mannose, maltose, sucrose, and rhamnose contributed minimally (VIP < 1) (Fig. 4d). Metabolites with VIP values greater than 1 are generally considered significant biomarkers contributing to group separation (Zhang et al., 2025). Several high-VIP phenolic acids and flavonoids identified in this study are widely recognized for their bioactive and health-promoting properties, suggesting that these metabolites contribute substantially to the functional and nutraceutical potential of *Syzygium cumini*. Similar metabolomics studies have reported key discriminatory metabolites in fruits such as citrus⁵², cashew apple⁴⁷, and Gannan oranges⁵³, supporting the utility of VIP analysis in identifying tissue-specific phytochemical biomarkers. The VIP score thus gives insight into the pivotal metabolites associated with tissue-specific metabolic variation in jamun.

Antioxidant and α -amylase inhibition activities

The antioxidant (DPPH) and α -amylase inhibition activities of pulp and seed extracts from three jamun genotypes are presented in Table 5. Significant differences ($p < 0.05$) were observed across genotypes and tissues.

DPPH radical scavenging activity

Seed extracts demonstrated markedly higher scavenging activity than pulp extracts. The strongest activity was recorded in PCJ-15 seed ($96.82 \pm 0.38\%$), followed by PCJ-15 pulp ($92.84 \pm 1.36\%$). Moderate activity was observed in PCJ-9 seed ($54.14 \pm 0.69\%$), while the lowest values occurred in the pulp samples of PCJ-17 ($20.17 \pm 0.63\%$). The IC_{50} values further confirmed this trend. The most potent antioxidant extracts were PCJ-15 seed ($38.57 \pm 0.65 \mu\text{g/mL}$) and PCJ-15 pulp ($40.53 \pm 1.17 \mu\text{g/mL}$), both of which were significantly lower than the other samples (Table 5).

This trend of seed > pulp antioxidant activity has been consistently reported across multiple studies on *Syzygium cumini*. seeds typically contain higher concentrations of gallic acid, ellagic acid, catechins, and tannins, potent hydrogen-donating phenolics responsible for stronger free-radical-scavenging capacity^{53,32}. These compounds possess multiple hydroxyl groups that enhance electron-transfer and proton-donation reactions, explaining the lower IC_{50} values in seed extracts⁵⁵. Genotype-dependent variation in DPPH activity, as observed between PCJ-9, PCJ-15 and PCJ-17, is also consistent with earlier reports showing that phenolic accumulation in jamun fruit parts varies substantially among landraces⁵⁴. Smaller-fruited genotypes often possess more concentrated phenolic metabolites per gram of tissue due to reduced dilution across fruit biomass, which may account for the superior activity of PCJ-15⁵⁶. Thus, the strong DPPH activity and low IC_{50} values in PCJ-15, particularly in seeds, can be attributed to its richer flavonoids profile and higher antioxidant density compared with the larger-fruited genotypes.

α -amylase inhibition activity

A parallel pattern was observed for α -amylase inhibition. The highest inhibitory activity was found in PCJ-15 seed ($96.85 \pm 1.00\%$), followed by PCJ-15 pulp ($85.56 \pm 0.97\%$). Moderate inhibition occurred in PCJ-9 seed ($67.66 \pm 1.23\%$), whereas the lowest values were recorded in PCJ-17 pulp ($40.55 \pm 0.74\%$). IC_{50} trends aligned with inhibition percentages. The lowest IC_{50} values, indicating the strongest antidiabetic potential, were observed in PCJ-15 seed ($38.57 \pm 0.65 \mu\text{g/mL}$) and PCJ-15 pulp ($40.53 \pm 1.17 \mu\text{g/mL}$). Higher IC_{50} values in PCJ-9 seed ($69.03 \pm 0.75 \mu\text{g/mL}$) and PCJ-17 seed ($116.69 \pm 3.26 \mu\text{g/mL}$) indicated moderate potency (Table 5).

Seed and kernel fractions of jamun exhibit the highest α -amylase inhibition, whereas pulp shows comparatively weaker activity, owing to the higher concentration of phenolics, flavonoids, tannins, glycosides and saponins in seed-derived tissues⁵⁴. Their observation of markedly lower IC_{50} values in seed and kernel extracts aligns closely with our results, where PCJ-15 seed ($38.57 \mu\text{g/mL}$) and PCJ-15 pulp ($40.53 \mu\text{g/mL}$) recorded the strongest inhibitory effects among the evaluated genotypes. Phenolics inhibit α -amylase through hydrogen bonding and hydrophobic interactions with catalytic residues, reducing enzyme activity⁵⁵. Moreover, previous studies have shown that natural inhibitors from jamun seed act through suppression of pancreatic α -amylase activity, thereby reducing starch hydrolysis and postprandial glucose spikes⁵⁷⁻⁵⁸. This mechanistic explanation aligns closely with the strong inhibitory effect observed in PCJ-15 and the moderate inhibition in PCJ-9 and PCJ-17 seed extracts. Thus, our findings highlight genotype-dependent variability in α -amylase inhibition, emphasizing the potential of jamun phytochemicals as effective natural antidiabetic agents with significant nutraceutical relevance.

Correlation between phytochemical composition and biological activities

Pearson correlation analysis was performed to explore the relationships among key phytochemicals, antioxidant activity, and α -amylase inhibition (Fig. 5). Total flavonoid content (TFC) exhibited very strong positive correlations with

DPPH scavenging activity ($r = 0.97$) and α -amylase inhibition ($r = 0.95$), suggesting that flavonoids represent the major functional component responsible for the antioxidant and antidiabetic potential of the extract. In contrast, total phenolic content (TPC) showed relatively weak positive correlations with DPPH activity ($r = 0.10$) and α -amylase inhibition ($r = 0.12$), indicating that specific flavonoid subclasses, rather than the total phenolic pool, are the primary drivers of bioactivity in this matrix. This aligns with previous reports highlighting the superior radical-scavenging and enzyme-inhibitory capacities of flavonoids compared with other phenolic classes⁵⁹. Among the identified phenolic acids, protocatechuic acid ($r = 0.69$ and $r = 0.75$) and ferulic acid ($r = 0.68$ and $r = 0.74$) showed the strongest positive correlations with DPPH radical scavenging activity and α -amylase inhibition, respectively. Sinapic acid also showed prominent contributions ($r = 0.62$ and $r = 0.70$), followed by 2,4-dihydroxybenzoic acid ($r = 0.56$ and $r = 0.64$) and caffeic acid ($r = 0.56$ and $r = 0.66$). In contrast, vanillic acid showed strong negative correlations with both DPPH activity ($r = -0.89$) and enzyme inhibition ($r = -0.84$), suggesting its presence was inversely related to the overall bioactive potency. These findings align with recent studies highlighting the structural influence of phenolic acid derivatives on enzyme inhibition and free radical neutralization⁶⁰⁻⁶¹. The strong antioxidant activity of phenolic compounds is largely attributed to the presence of multiple hydroxyl groups, which enhance their radical-scavenging and electron-donating capacities. Moreover, hydroxylated cinnamates are better scavengers than vanillic acid⁶². Among the individual flavonoids, catechin emerged as a primary contributor, showing strong positive correlations with both DPPH radical scavenging activity ($r = 0.84$) and α -amylase inhibition ($r = 0.76$). Significant associations were also observed for hesperetin ($r = 0.83$ and $r = 0.75$) and rutin ($r = 0.56$ and $r = 0.66$, respectively), while myricetin ($r = 0.55$ and $r = 0.43$) exhibited moderate correlations. Collectively, these results indicate that the synergistic effects of multiple flavonoid compounds likely drive the observed bioactivities. Flavan-3-ols and flavonols such as catechin, rutin, and myricetin are well known for their strong antioxidant capacity and ability to inhibit carbohydrate-hydrolyzing enzymes. This activity is largely attributed to their multiple hydroxyl groups and favourable structural configuration, which enhance radical scavenging and enzyme-binding interactions. Similar associations between these flavonoids and DPPH scavenging as well as α -amylase inhibitory activity have been widely reported in phytochemical and nutraceutical studies⁶³⁻⁶⁴. Total anthocyanin content (TAC) displayed moderate correlations with DPPH activity ($r = 0.56$) and α -amylase inhibition ($r = 0.44$), with its individual components delphinidin ($r = 0.61$ and $r = 0.49$) and cyanidin ($r = 0.47$ and $r = 0.36$) following a similar trend. Similarly, studies on diverse herbal infusions and plant extracts report that global antioxidant and α -amylase/ α -glucosidase inhibitory effects correlate more tightly with total phenolic or flavonoid content than with anthocyanins alone, suggesting that anthocyanins usually play a secondary or complementary role⁶⁵⁻⁶⁶. A very strong positive correlation was observed between DPPH scavenging activity and α -amylase inhibition ($r = 0.98$), suggesting that the same phytochemical constituents, particularly flavonoids and specific phenolic acids, simultaneously contribute to both antioxidant and enzyme inhibitory activities. Furthermore, IC_{50} values showed strong negative correlations with TFC ($r = -0.83$), DPPH activity ($r = -0.83$), and α -amylase inhibition ($r = -0.87$), and a moderate negative correlation with TPC ($r = -0.46$), confirming that samples with higher phytochemical accumulation require lower concentrations to achieve 50% inhibition. These findings highlight the central role of polyphenolic compounds in determining the antioxidant and α -amylase inhibitory potential of *Syzygium cumini*, supporting its value as a functional fruit rich in bioactive phytochemicals with antioxidant and antidiabetic properties.

KEGG pathway analysis of phytochemical biosynthesis in *Syzygium cumini*

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway metabolite network analysis revealed that the detected metabolites in jamun pulp and seed samples were highly interconnected with secondary metabolite biosynthesis and carbohydrate metabolism (Fig. 6). Flavonoid biosynthesis emerged as a key metabolic hub, involving metabolites such as quercetin, kaempferol, luteolin, and epicatechin, which are well known for their antioxidant and enzyme

inhibitory activities. These compounds are biosynthetically linked to the phenylpropanoid pathway through intermediates such as trans-cinnamate and p-coumarate, highlighting the importance of phenylpropanoid metabolism in shaping the phytochemical composition of *Syzygium cumini*. The enrichment of flavonoid and phenylpropanoid pathways supports previous genomic and biochemical studies indicating that jamun possesses a highly active secondary metabolite system responsible for its medicinal and nutraceutical properties⁶⁷⁻⁶⁸. In addition to secondary metabolism, galactose metabolism emerged as the core of the carbohydrate cluster, connecting major sugars such as glucose, fructose, sucrose, and mannose with pathways including starch/sucrose metabolism and fructose/mannose pathways. The predominance of glucose and fructose in pulp tissues reflects the accumulation of soluble sugars associated with fruit ripening and sweetness. In contrast, seed tissues exhibited relatively stronger connections with starch metabolism, indicating their role as carbohydrate storage reservoirs. Such metabolic partitioning between pulp and seed tissues reflects source–sink dynamics during fruit development and contributes to the nutritional and functional attributes of jamun fruits⁶⁹⁻⁷⁰. Most sugars (glucose, fructose, maltose, trehalose, sucrose) formed a negatively correlated cluster with phenolics and flavonoids, indicating an inverse relationship between primary carbohydrate accumulation and secondary metabolite synthesis (Fig. 7). This trend was most pronounced for fructose and sucrose, which showed strong negative correlations ($r < -0.70$) with compounds like caffeic acid, ferulic acid, and quercetin. The metabolic divergence is further supported by previous studies in apple cultivars, where citric acid (associated with the metabolism of phenolics) showed negative correlations with fructose, glucose, and sucrose⁷¹. This inverse metabolic allocation identifies *Syzygium cumini* as a naturally optimized delivery system for high-density phytochemicals with minimal glycemic impact. Consequently, these metabolic insights establish a robust mechanistic foundation for the clinical application of jamun-derived bioactives in the prevention and management of chronic oxidative and metabolic disorders.

Conclusions

This study provides a high-resolution metabolomic blueprint of *Syzygium cumini* (L.) Skeels, demonstrating that its therapeutic efficacy is driven by specific flavonoid subclasses rather than total phenolic concentration. By mapping the metabolic partitioning between pulp and seed, we identified catechin and protocathechuic acid as the fundamental drivers of antioxidant and α -amylase inhibitory mechanisms. The discovery of an inverse correlation between sugar accumulation and secondary metabolite synthesis highlights the PCJ-15 genotype as a potent, low-glycemic phytochemical source. Our KEGG-based pathway mapping establishes a clear biosynthetic link between phenylpropanoid metabolism and bioactivity. Ultimately, these findings provide the mechanistic evidence required to transition *S. cumini* from traditional medicine to a standardized clinical candidate.

Materials and methods

Plant material and genotype selection

Three contrasting *Syzygium cumini* (jamun) genotypes, PCJ-9, PCJ-17, and PCJ-15, were selected based on our previously characterised morpho-biochemical and bioactive diversity⁷². Among them, PCJ-9 and PCJ-17 represent large-fruited, superior genotypes, whereas PCJ-15 represented a small-fruited, contrasting genotype characterized by a higher seed proportion and elevated phenolic and flavonoid content. Fully ripened fruits were collected from healthy trees of uniform age and growth conditions from the experimental site at ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. Fruits were washed, manually separated into pulp and seed, immediately frozen in liquid nitrogen, and stored at -80°C until metabolite extraction and LC–MS/MS analysis.

Determination of phenolic acids and flavonoids by LC–MS/MS

Extraction procedure

Phenolic acids and flavonoids were extracted by an altered procedure^{73–74}. In short, 80% methanol was used to extract freeze-dried powdered sample, then sonication for 20 min was applied and the centrifugation at $5,000 \times g$ for 10 min at 4°C was performed; the residue was extracted two more times and the pooled supernatants were evaporated under vacuum at a temperature of 45°C and then dissolved again in 5 mL of acidified aqueous methanol (0.1% formic acid) to get the free phenolic fraction. The solution was twice washed with n-hexane in order to waste the lipophilic impurities, and the aqueous phase underwent a filter (0.22 µm PTFE) for LC–MS/MS analysis. To find out bound phenolics, the residue that was left after methanolic extraction was hydrolyzed with 2 mL of 2 M NaOH under nitrogen for 2 hours at room temperature, acidified to pH 2.0 with 6 M HCl, and then ethyl acetate was used to extract three times; the ethyl acetate phase that was separated was evaporated ($\leq 40^\circ\text{C}$), and the residue was dissolved in 1 mL of LC–MS-grade methanol and filtered before injection. Free (non-hydrolyzed) extracts were taken for the quantification of native flavonoid glycosides, while alkaline hydrolysates were utilized solely for bound phenolics.

LC–MS/MS conditions

Chromatographic separation was performed on an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm; Waters, USA) equipped with a Vanguard pre-column. The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. A linear gradient was applied at a flow rate of 0.1 mL min⁻¹, and the column temperature was maintained at 25°C. The injection volume was 6 µL. Online detection was achieved using a photodiode array (PDA) detector coupled to a TQD triple-quadrupole mass spectrometer (Waters, USA) equipped with an electrospray ionization source (ESI). Quantification was performed in multiple reaction monitoring (MRM) mode using optimized precursor–product ion transitions for each analyte.

Anthocyanin analysis by LC–MS/MS

Extraction procedure

The extraction of anthocyanins was done according to a modified protocol⁷⁵. In short, a sample of 1.5 g was finely homogenized and mixed with 20 mL of methanol containing 1% (v/v) HCl and incubated in the dark at 4°C for 12 h with shaking at intervals. The extract was spun down ($10,000 \times g$, 10 min, 4°C) and the supernatant was taken. A portion of 1 mL from the liquid on top was evaporated to a dry state under reduced pressure at a temperature of 30°C or less and was dissolved in 1 mL of the initial LC mobile phase. Before LC–MS/MS analysis, the sample was filtered using a 0.22 µm PTFE syringe filter to eliminate particulates.

LC–MS/MS conditions

The separation of anthocyanins using chromatography was carried out on an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm; Waters, USA) outfitted with a Vanguard pre-column. The solvents were (A) water with 0.25% formic acid and (B) acetonitrile with 0.25% formic acid. The elution was carried out by using a linear gradient and a flow rate of 0.25 mL min⁻¹. The column was heated to 35°C, and the volume for injection was 6 µL. The ACQUITY TQD triple quadrupole mass spectrometer (Waters, USA) was used for the detection and quantification of the anthocyanins.

Sugar analysis by LC–MS/MS

Extraction procedure

The technique for sugar extraction here has been adapted from Steppuhn and Wäckers⁷⁶ through modification. The sample was powdered, and its exact weight was taken, then the extraction was done twice with warm 80% ethanol while gentle vortex stirring. The supernatant obtained from the pooling was centrifuged ($10,000 \times g$, 10 min), and then it was diluted to 20 mL with 80% ethanol. A 1 mL portion was dried under reduced pressure at $\leq 40^\circ\text{C}$ to prevent thermal degradation of sugars and the residue was dissolved in 0.5 mL of ultrapure water with 0.1% formic acid. The mixture was vortexed for 1 min, sonicated for 5 min, and then filtered using a $0.22 \mu\text{m}$ nylon membrane. There was no ethyl acetate partitioning done because polar sugars are just in the aqueous phase. The filtrate was then diluted with the mobile phase (solvents A and B, 1:1 v/v) as needed and injected into the LC–MS/MS system.

LC–MS/MS conditions

The chromatographic separation of the soluble sugars was implemented on an Acquity UPLC BEH Amide column having a dimension of $2.1 \times 100 \text{ mm}$ with $1.7 \mu\text{m}$ particle size (Waters, USA) and equipped with a BEH Amide Vanguard pre-column. The mobile phases comprised: (A) acetonitrile and (B) 10 mM ammonium acetate solution in water (pH 4.5), both of which were filtered over ($0.22 \mu\text{m}$). A linear HILIC gradient was applied at a flow rate of 0.30 mL min^{-1} : 90% A (0–1 min), 90–70% A (1–10 min), 70–50% A (10–13 min), 50–90% A (13–15 min), followed by re-equilibration at 90% A (15–18 min). The temperature of the column was held at 35°C , and the volume of the sample to be injected was $2 \mu\text{L}$. The detection was enabled using a photodiode array (PDA) detector that was coupled in-line to a TQD triple-quadrupole mass spectrometer (Waters, USA), which operated under multiple reaction monitoring (MRM) mode with the use of electrospray ionization. The identification and quantification of carbohydrates were based on external calibration curves of the respective authentic standards, using the optimized precursor/product ion transitions.

Instrumentation and standards

All chromatographic analyses were performed on a Waters ACQUITY UPLC-H Class system (Waters, USA) connected to a TQD triple-quadrupole tandem mass spectrometer with an electrospray ionization (ESI) source. Only LC–MS-grade solvents were used for both sample preparation and mobile phases. Phenolic acid and flavonoid standards ($\geq 98\%$ purity) were sourced from Sigma-Aldrich (USA), and the quantitative analysis was done with the help of external calibration curves made from the corresponding standards. Mass spectrometric detection was performed in negative electrospray ionization (ESI[−]) mode, monitoring the predominant deprotonated molecular ions $[\text{M} - \text{H}]^-$. These precursor ions were subjected to compound-specific collision energies to generate characteristic product ions, which were quantified using Multiple Reaction Monitoring (MRM). Detailed MRM parameters and representative chromatograms of sample extracts are provided in the supplementary information (Table S1–S4; Figs. S1–S8).

DPPH radical scavenging activity

The antioxidant capacity of the extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay following the procedure⁷⁷ with modifications as applied in earlier work on jamun biochemical profiling⁷². A DPPH stock solution was prepared by dissolving 25 mg of DPPH in 100 mL of methanol and adjusting the absorbance to 0.70 ± 0.01 at 517 nm. For sample extraction, 2 g of jamun fruit tissue was homogenized in 10 mL methanol and centrifuged at $10,000 \times g$ for 20 min. An aliquot of $100 \mu\text{L}$ of the resulting supernatant was combined with 3.9 mL of the DPPH working solution and incubated in the dark for 2 h at room temperature. The decrease in absorbance at 517 nm was recorded against a reagent blank, and the percentage of DPPH radical scavenging was calculated by comparing the absorbance of the reaction mixture with the control.

$$\text{DPPH Inhibition (\%)} = \left\{ \frac{A_c - A_s}{A_c} \right\} \times 100$$

Where, A_c = Absorbance (optical density) of DPPH

A_s = Absorbance of sample

α -amylase inhibitory activity

The α -amylase inhibitory activity of fruit extracts was evaluated using porcine pancreatic α -amylase (PPA; EC 3.2.1.1) following the 3,5-dinitrosalicylic acid (DNSA) colorimetric method described by Miller⁷⁸ and as adopted in jamun antidiabetic studies by Gajera et al.⁵⁴. Briefly, 200 μ L of extract (0.1–300 μ g/mL for IC_{50} determination) was pre-incubated with 200 μ L of PPA solution (1 U/mL in 0.02 M phosphate buffer, pH 6.9, containing 0.006 M NaCl) at 37°C for 10 min. The reaction was initiated by adding 200 μ L of 1% (w/v) soluble starch, followed by incubation for 15 min at 37°C. The reaction was terminated with 200 μ L DNSA reagent, and the mixture was heated at 100°C for 5 min, cooled, diluted with 2 mL distilled water, and the absorbance was measured at 540 nm. Enzyme control (without extract), sample color control, and reagent blank were included, while acarbose served as the positive standard. Percent inhibition was calculated after blank correction using: The IC_{50} value, defined as the extract concentration required to inhibit 50% of PPA activity, was estimated from nonlinear regression of inhibition (%) versus log concentration. One unit of enzyme activity corresponded to the amount of enzyme releasing 1 μ mol of maltose per minute under assay conditions

Statistical analysis

A two-way analysis of variance (ANOVA) was performed on quantitative LC–MS/MS data to evaluate the impact of genotype and tissue type, along with their interaction. The threshold for statistical significance was set at $p < 0.05$. The results are reported as mean $\pm$ standard deviation (SD) based on three independent biological replicates ($n = 3$). The RAISINS statistical environment⁷⁹ was used for significance testing and graphical visualization. Multivariate analyses such as hierarchical clustering analysis (HCA), principal component analysis (PCA), correlation network analysis, and orthogonal partial least squares-discriminant analysis (OPLS-DA), KEGG pathway analysis were performed using MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca>)⁸⁰ and the Metware Biotechnology (MetwareBio) analytical platform (<https://www.metwarebio.com>). Identified metabolites were mapped to metabolic pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<http://www.kegg.jp/kegg/kegg1.html>)^{81–83}. Formal permission for the use and adaptation of KEGG pathway imagery was obtained from Kanehisa Laboratories, and the results were used to visualize metabolite-pathway interaction networks.

Declarations

Competing interests

The authors declare no competing interests.

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Author Contribution

****Ajay Kumar:**** Conceptualization; Methodology; Investigation; Data curation; Formal analysis; Validation; Visualization; Writing – original draft; Resources. ****Kanhaiya Singh:**** Conceptualization; Methodology; Validation; Formal analysis; Resources; Supervision; Visualization; Writing – review & editing; Project administration. ****Jai Prakash** :** Conceptualization; Methodology; Supervision; Project administration; Validation; Writing – review & editing. ****Amit Kumar Goswami:**** Methodology; Investigation; Resources; Validation; Writing – review & editing. ****Vishaw Bandhu Patel:**** Conceptualization; Methodology; Investigation; Formal analysis; Visualization; Supervision; Writing – review & editing. ****Amit Kumar Singh:**** Methodology; Resources; Supervision; Writing – review & editing. ****Yamini Yadav:**** Investigation; Formal analysis; Visualization ****;**** Writing – review & editing. ****Aditya Dnyaneshwar Ingole:**** Investigation; Data curation; Resources. ****Bhupendra Sagore:**** Investigation; Resources; Data curation.

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Data Availability

Data will be made available on request.

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Tables

Table 1. Concentration of individual phenolic acids ($\mu\text{g g}^{-1}$ FW) in pulp and seed tissues of three jamun (*Syzygium cumini* (L.) Skeels) genotypes.

Compound	Tissue & Genotypes					
	Pulp			Seed		
	PCJ-9	PCJ-17	PCJ-15	PCJ-9	PCJ-17	PCJ-15
Benzoic acid	483.07 ± 6.83 ^b	451.67 ± 0.12 ^c	660.14 ± 5.60 ^a	283.32 ± 11.01 ^a	272.48 ± 0.74 ^c	280.76 ± 3.31 ^b
p hydroxy benzoic acid	0.79 ± 0.02 ^a	0.78 ± 0.01 ^a	0.75 ± 0.00 ^b	0.45 ± 0.01 ^c	0.66 ± 0.02 ^b	1.46 ± 0.03 ^a
Salicylic acid	0.35 ± 0.01 ^a	0.21 ± 0.01 ^b	0.22 ± 0.00 ^b	0.29 ± 0.00 ^a	0.22 ± 0.01 ^b	0.18 ± 0.00 ^c
3 Hydroxy benzoic acid	2.75 ± 0.02 ^a	1.77 ± 0.04 ^b	1.34 ± 0.01 ^c	0.59 ± 0.07 ^c	1.70 ± 0.02 ^a	1.08 ± 0.05 ^b
t Cinnamic acid	8.13 ± 0.10 ^a	0.83 ± 0.01 ^c	6.00 ± 0.11 ^b	2.81 ± 0.05 ^a	2.18 ± 0.01 ^b	0.92 ± 0.01 ^c
2,4 dihydroxy benzoic acid	0.82 ± 0.03 ^a	0.65 ± 0.03 ^b	0.52 ± 0.05 ^c	0.44 ± 0.03 ^b	0.52 ± 0.04 ^b	2.83 ± 0.08 ^a
Gentisic acid	0.65 ± 0.02 ^b	0.08 ± 0.01 ^c	1.18 ± 0.01 ^a	0.12 ± 0.01 ^b	0.24 ± 0.01 ^a	0.13 ± 0.00 ^b
Protocatechuic acid	0.02 ± 0.00 ^b	0.01 ± 0.00 ^c	0.07 ± 0.00 ^a	0.02 ± 0.00 ^b	0.02 ± 0.00 ^b	0.59 ± 0.02 ^a
p Coumaric acid	4.54 ± 0.00 ^a	1.64 ± 0.03 ^c	3.66 ± 0.02 ^b	0.22 ± 0.01 ^c	2.54 ± 0.01 ^a	1.04 ± 0.01 ^b
o Coumaric acid	3.39 ± 0.02 ^a	1.01 ± 0.03 ^c	2.35 ± 0.02 ^b	0.13 ± 0.00 ^b	0.08 ± 0.00 ^c	2.12 ± 0.01 ^a
Vanillic acid	12.41 ± 0.02 ^b	14.33 ± 0.05 ^a	5.35 ± 0.07 ^c	12.63 ± 0.12 ^a	10.37 ± 0.01 ^b	8.12 ± 0.11 ^c
Gallic acid	310.20 ± 1.07 ^b	223.68 ± 0.37 ^c	313.58 ± 1.92 ^a	919.73 ± 4.10 ^a	830.05 ± 0.80 ^b	492.44 ± 1.03 ^c
Caffeic acid	0.15 ± 0.01 ^a	0.06 ± 0.01 ^c	0.08 ± 0.00 ^b	0.06 ± 0.00 ^b	0.06 ± 0.00 ^c	0.32 ± 0.00 ^a
Ferulic acid	0.10 ± 0.00 ^b	0.05 ± 0.00 ^c	0.33 ± 0.00 ^a	0.10 ± 0.00 ^c	0.57 ± 0.01 ^b	1.26 ± 0.04 ^a
Syringic acid	2.62 ± 0.02 ^a	0.73 ± 0.03 ^c	1.43 ± 0.01 ^b	0.96 ± 0.02 ^b	1.06 ± 0.02 ^a	0.37 ± 0.02 ^c
Sinapic acid	0.09 ± 0.00 ^b	0.07 ± 0.00 ^c	0.13 ± 0.00 ^a	0.10 ± 0.00 ^b	0.21 ± 0.02 ^b	22.67 ± 0.37 ^a
Ellagic acid	1.16 ± 0.11 ^c	16.54 ± 0.47 ^b	31.96 ± 1.33 ^a	396.02 ± 3.11 ^a	2.44 ± 0.24 ^c	10.23 ± 1.14 ^b
Chlorogenic acid	0.01 ± 0.00 ^c	0.05 ± 0.00 ^b	1.58 ± 0.00 ^a	0.01 ± 0.00 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b

*Mean ± SD (n=3) Values with different letters within the same evaluation of same tissue are significantly different (p<0.05)

Table 2. Concentration of individual flavonoids (ng g⁻¹ FW) in pulp and seed tissues of three jamun (*Syzygium cumini* (L.) Skeels) genotypes.

Compound	Tissue & Genotypes					
	Pulp			Seed		
	PCJ-9	PCJ-17	PCJ-15	PCJ-9	PCJ-17	PCJ-15
Umbelliferone	1.67 ± 0.27 ^a	1.28 ± 0.07 ^b	1.37 ± 0.15 ^b	2.42 ± 0.12 ^a	2.34 ± 0.04 ^a	1.27 ± 0.13 ^b
Apigenin	10.93 ± 1.30 ^b	18.41 ± 1.70 ^a	19.05 ± 0.62 ^a	27.07 ± 3.32 ^a	9.20 ± 0.51 ^b	9.67 ± 1.09 ^b
Galangin	1.44 ± 0.06 ^b	1.45 ± 0.09 ^b	2.67 ± 0.26 ^a	2.06 ± 0.12 ^a	1.59 ± 0.16 ^b	1.16 ± 0.15 ^c
Naringenin	128.59 ± 11.02 ^b	169.05 ± 5.71 ^a	142.44 ± 4.97 ^b	3232.25 ± 97.15 ^a	1619.83 ± 11.57 ^c	2913.85 ± 77.10 ^b
Kaemperol	2.06 ± 0.09 ^b	4.73 ± 0.33 ^a	2.27 ± 0.19 ^b	14.99 ± 0.24 ^a	1.44 ± 0.18 ^c	2.59 ± 0.09 ^b
Luteolin	170.91 ± 11.28	178.45 ± 7.14	151.85 ± 16.27	101.54 ± 14.07 ^c	137.94 ± 9.81 ^b	232.77 ± 15.75 ^a
Fisetin	0.17 ± 0.02 ^c	1.44 ± 0.11 ^b	2.60 ± 0.09 ^a	0.18 ± 0.01 ^a	0.10 ± 0.01 ^b	0.08 ± 0.00 ^c
Eriodictyol	0.72 ± 0.02 ^a	0.23 ± 0.02 ^b	0.22 ± 0.02 ^b	0.28 ± 0.02 ^c	2.00 ± 0.12 ^a	0.50 ± 0.02 ^b
Catechin	870.00 ± 17.37 ^b	217.98 ± 9.12 ^c	6046.70 ± 203.76 ^a	69.46 ± 6.44 ^c	1164.72 ± 43.96 ^b	3421.79 ± 296.15 ^a
Epicatechin	237.88 ± 11.86 ^c	416.88 ± 28.47 ^b	523.54 ± 26.74 ^a	1014.21 ± 38.15 ^b	3029.87 ± 24.80 ^a	733.39 ± 10.08 ^c
Hesperetin	112.72 ± 3.30 ^c	219.45 ± 14.30 ^b	696.33 ± 20.96 ^a	407.93 ± 40.41 ^b	46.71 ± 2.18 ^c	480.50 ± 15.04 ^a
Quercetin	380.16 ± 25.20 ^b	530.20 ± 13.74 ^a	341.71 ± 20.89 ^b	401.13 ± 8.54 ^b	221.78 ± 6.88 ^c	695.51 ± 10.86 ^a
Epigallocatechin	213.72 ± 14.03 ^b	766.12 ± 26.29 ^a	157.09 ± 12.02 ^c	5720.11 ± 113.39 ^a	91.98 ± 8.23 ^c	3479.17 ± 164.23 ^b
Myricetin	3454.74 ± 327.23 ^b	384.10 ± 22.78 ^c	27111.70 ± 48.53 ^a	1959.91 ± 64.10 ^a	637.93 ± 9.68 ^c	1002.67 ± 22.42 ^b
Rutin	831.38 ± 14.95 ^a	178.93 ± 4.61 ^b	98.75 ± 6.35 ^c	10369.12 ± 40.25 ^b	3230.42 ± 92.51 ^c	20331.19 ± 364.36 ^a

*Mean ± SD (n=3) Values with different letters within the same evaluation of same tissue are significantly different (p<0.05)

Table 3. Concentration of individual anthocyanins (µg g⁻¹ FW) in pulp and seed tissues of three jamun (*Syzygium cumini* (L.) Skeels) genotypes.

Compound	Tissue & Genotypes					
	Pulp			Seed		
	PCJ-9	PCJ-17	PCJ-15	PCJ-9	PCJ-17	PCJ-15
Pelargonidin	0.26 ± 0.03 ^b	0.32 ± 0.02 ^a	0.16 ± 0.02 ^c	0.48 ± 0.02 ^a	0.12 ± 0.02 ^b	0.08 ± 0.01 ^c
Cyanidin	296.85 ± 7.34 ^b	6.27 ± 0.37 ^c	1076.03 ± 26.30 ^a	28.36 ± 1.75 ^a	3.30 ± 0.33 ^b	2.95 ± 0.29 ^b
Delphinidin	363.39 ± 12.49 ^b	58.31 ± 0.90 ^c	2601.55 ± 119.29 ^a	387.10 ± 19.76 ^a	292.52 ± 10.57 ^c	333.43 ± 12.28 ^b
Malvidin	168.86 ± 1.25 ^a	12.02 ± 0.33 ^c	135.03 ± 3.03 ^b	10.43 ± 0.15 ^a	0.55 ± 0.04 ^b	0.24 ± 0.08 ^c

*Mean ± SD (n=3) Values with different letters within the same evaluation of same tissue are significantly different (p<0.05)

Table 4. Concentration of individual sugars (µg g⁻¹ FW) in pulp and seed tissues of three jamun (*Syzygium cumini* (L.) Skeels) genotypes.

Compound	Tissue & Genotypes					
	Pulp			Seed		
	PCJ-9	PCJ-17	PCJ-15	PCJ-9	PCJ-17	PCJ-15
Ribose	46.26 ± 1.08 ^b	62.55 ± 0.52 ^a	41.22 ± 0.70 ^c	8.06 ± 0.02 ^a	5.54 ± 0.08 ^c	6.45 ± 0.04 ^b
Arabinose	3.69 ± 0.24 ^a	3.16 ± 0.34 ^b	3.93 ± 0.19 ^a	1.91 ± 0.09	2.07 ± 0.11	2.03 ± 0.07
Xylose	1.24 ± 0.08 ^a	1.03 ± 0.02 ^b	0.83 ± 0.09 ^c	0.66 ± 0.01 ^b	0.73 ± 0.04 ^a	0.76 ± 0.04 ^a
Rhamnose	23.57 ± 0.40 ^a	2.35 ± 0.10 ^c	3.47 ± 0.17 ^b	8.84 ± 0.06 ^a	3.26 ± 0.11 ^b	2.58 ± 0.03 ^c
Fucose	10.85 ± 0.14 ^a	5.31 ± 0.27 ^c	10.18 ± 0.17 ^b	4.86 ± 0.07 ^b	3.75 ± 0.23 ^c	6.35 ± 0.07 ^a
Fructose	73960.67 ± 283.14 ^a	53278.19 ± 238.07 ^b	19567.64 ± 201.02 ^c	22841.97 ± 475.34 ^a	16240.59 ± 136.85 ^c	20948.60 ± 75.73 ^b
Glucose	15465.50 ± 200.26 ^a	11312.27 ± 44.59 ^c	12893.41 ± 30.38 ^b	4958.21 ± 80.78 ^a	3454.92 ± 40.26 ^c	4458.50 ± 8.96 ^b
Mannose	2690.56 ± 54.93 ^c	6326.92 ± 103.21 ^b	24372.95 ± 118.24 ^a	2818.02 ± 7.83 ^a	1886.95 ± 13.47 ^c	2517.64 ± 57.72 ^b
Inositol	423.88 ± 8.31 ^a	242.02 ± 3.66 ^b	213.52 ± 1.22 ^c	1028.04 ± 25.32 ^a	648.51 ± 12.44 ^c	982.45 ± 19.94 ^b
Sorbitol	208.35 ± 4.07 ^b	195.19 ± 1.19 ^c	224.17 ± 1.48 ^a	133.85 ± 3.94 ^c	156.92 ± 1.95 ^b	181.23 ± 0.88 ^a
Sucrose	45.80 ± 0.55 ^a	15.91 ± 0.21 ^b	8.94 ± 0.05 ^c	17.97 ± 0.08 ^c	117.53 ± 1.34 ^a	37.51 ± 0.63 ^b
Maltose	26.25 ± 0.43 ^a	11.61 ± 0.03 ^c	22.42 ± 0.11 ^b	16.69 ± 0.13 ^a	4.67 ± 0.07 ^c	12.17 ± 0.14 ^b

*Mean ± SD (n=3) Values with different letters within the same evaluation of same tissue are significantly different (p<0.05)

Table 5. Antioxidant (DPPH), α-amylase inhibition activities, and IC₅₀ values of pulp and seed extracts from three jamun (*Syzygium cumini* (L.) Skeels) genotypes.

Genotype	Tissue	DPPH Scavenging (%)	Inhibition (%)	IC50
PCJ-9	Pulp	33.93 ± 0.75 ^e	56.52 ± 0.73 ^e	133.85 ± 4.83 ^b
PCJ-9	Seed	54.14 ± 0.69 ^c	67.66 ± 1.23 ^c	69.03 ± 0.75 ^d
PCJ-15	Pulp	92.84 ± 1.36 ^b	85.56 ± 0.97 ^b	40.53 ± 1.17 ^e
PCJ-15	Seed	96.82 ± 0.38 ^a	96.85 ± 1.00 ^a	38.57 ± 0.65 ^e
PCJ-17	Pulp	20.17 ± 0.63 ^f	40.55 ± 0.74 ^f	302.20 ± 3.00 ^a
PCJ-17	Seed	41.52 ± 0.77 ^d	58.61 ± 0.97 ^d	116.69 ± 3.26 ^c
SE(d)		0.67	0.78	2.24
CV(%)		1.44	1.41	2.35

Values represent mean ± SD (n = 3). Different superscript letters within the same evaluation indicate significant differences at (p<0.05)

Figures

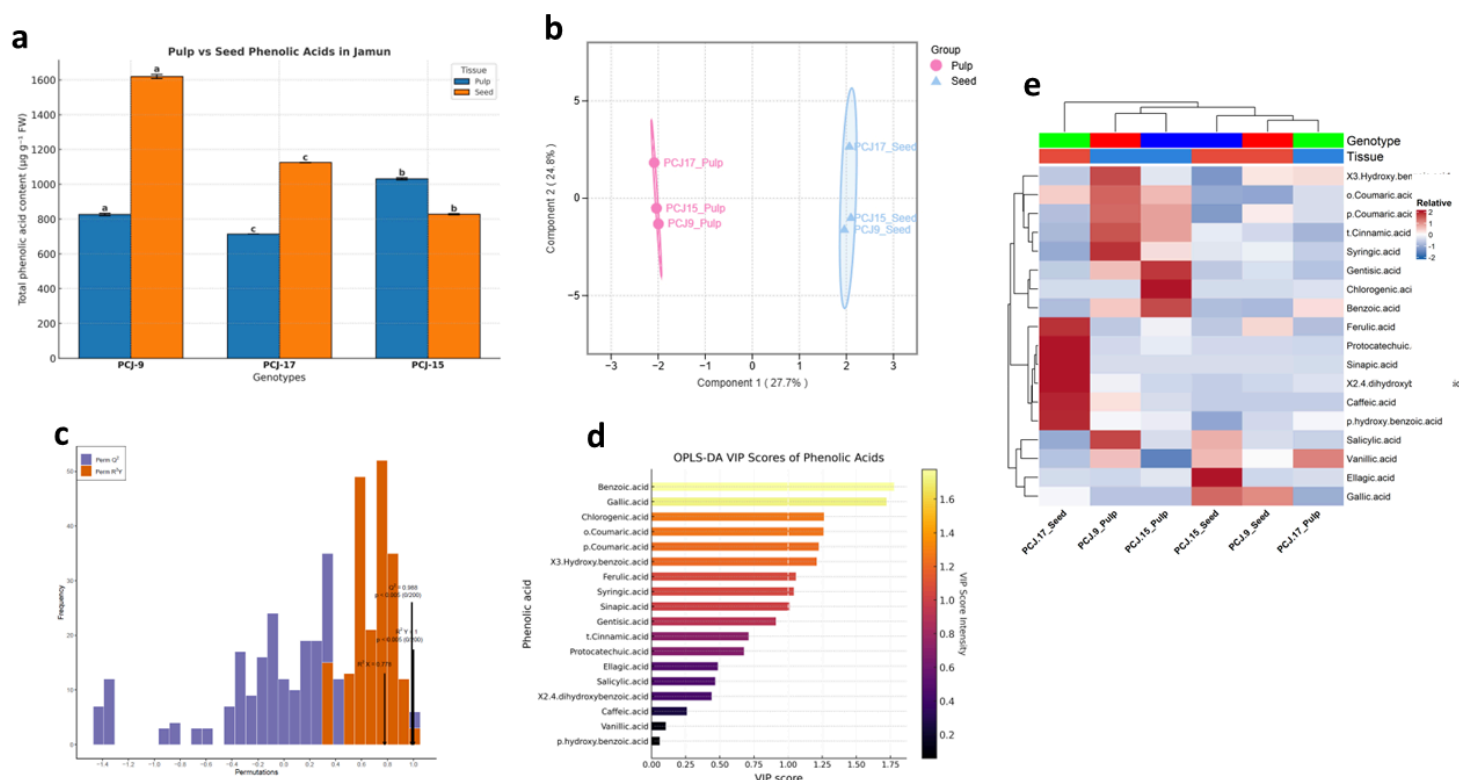


Figure 1

Comparative profiling of phenolic acids in jamun *Syzygium cumini*(L.) Skeels genotypes. (A) Total phenolic acid content (µg g⁻¹ FW) in pulp and seed tissues of three genotypes (PCJ-9, PCJ-17, and PCJ-15). Bars represent mean ± SD (n = 3); different letters indicate significant differences (p ≤ 0.05). (B) OPLS-DAScore plot showing separation

between pulp and seed samples. (C) OPLS-DA permutation test confirming model robustness ($R^2Y = 1.00$; $Q^2 = 0.988$; $p < 0.01$). (D) OPLS-DA VIP scores highlighting major discriminant phenolic acids. (E) Hierarchical heat map depicting relative abundance of individual phenolic acids across genotypes and tissues (red = high, blue = low).

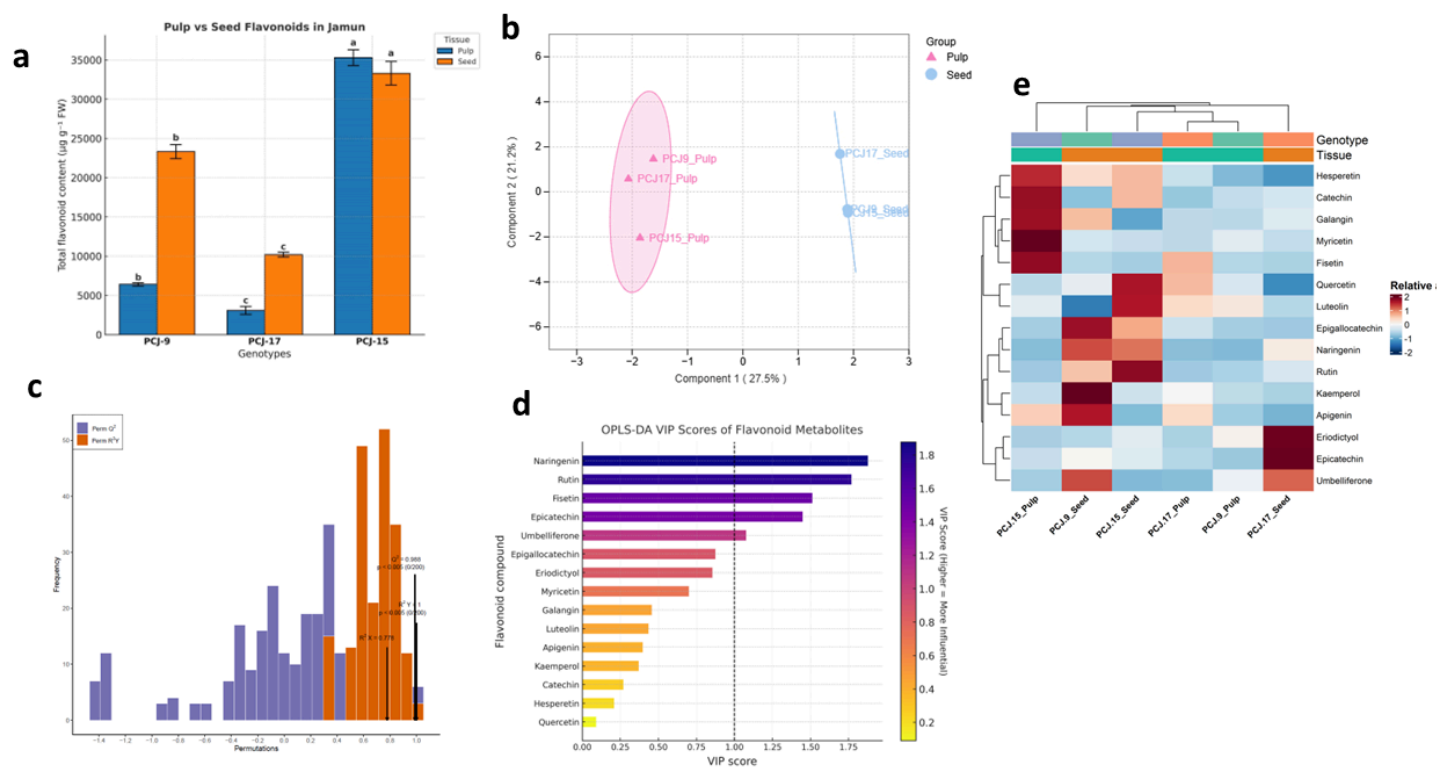


Figure 2

Comparative profiling of flavonoids in jamun *Syzygium cumini* (L.) Skeels genotypes. (A) Total flavonoid content ($\mu\text{g g}^{-1}$ FW) in pulp and seed tissues of three genotypes (PCJ-9, PCJ-17, and PCJ-15). Bars represent mean $\pm$ SD ($n = 3$); different letters indicate significant differences ($p \leq 0.05$). (B) OPLS-DA score plot showing clear differentiation of pulp and seed samples. (C) OPLS-DA permutation test validating model robustness ($R^2Y = 1.00$; $Q^2 = 0.988$; $p < 0.01$). (D) OPLS-DA VIP scores identifying major discriminant flavonoids. (E) Heat map clustering illustrating relative abundance of individual flavonoids across tissues (red = high, blue = low).

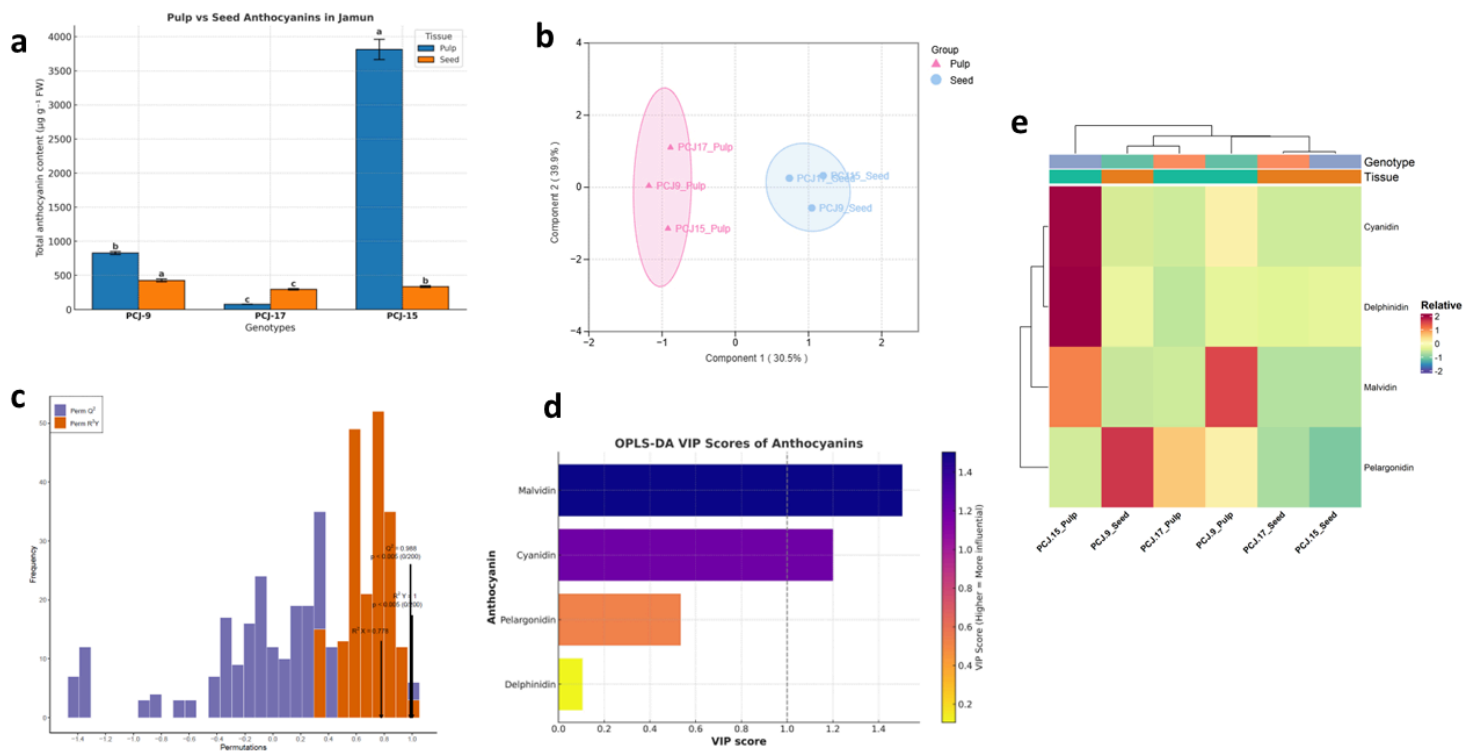


Figure 3

Comparative profiling of anthocyanins in jamun *Syzygium cumini* (L.) Skeels genotypes. (A) Total anthocyanin content ($\mu\text{g g}^{-1}$ FW) in pulp and seed tissues of three genotypes (PCJ-9, PCJ-17, and PCJ-15). Bars represent mean $\pm$ SD ($n = 3$); different letters indicate significant differences ($p \leq 0.05$). (B) OPLS-DA score plot showing distinct separation between pulp and seed samples based on anthocyanin profiles. (C) OPLS-DA permutation test validating model robustness ($R^2Y = 1.00$; $Q^2 = 0.988$; $p < 0.01$). (D) OPLS-DA VIP scores highlighting key discriminant anthocyanins contributing to class separation. (E) Heat-map clustering illustrating relative abundance of individual anthocyanins across genotypes and tissues (red = high, blue = low).

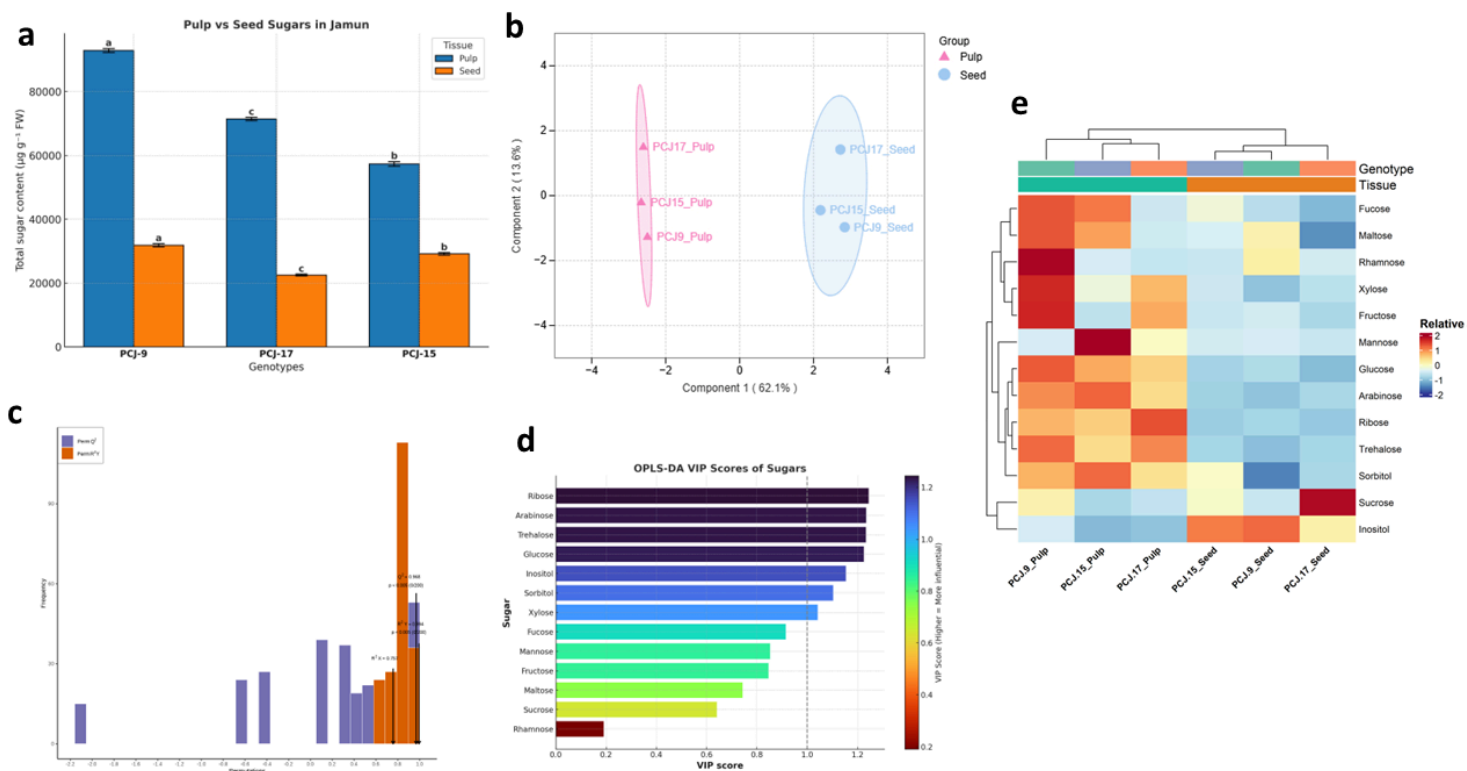


Figure 4

Comparative profiling of sugars in jamun *Syzygium cumini* (L.) Skeels genotypes. (A) Total sugar content ($\mu\text{g g}^{-1}$ FW) in pulp and seed tissues of three genotypes (PCJ-9, PCJ-17, and PCJ-15). Bars represent mean $\pm$ SD ($n = 3$); different letters indicate significant differences ($p \leq 0.05$). (B) OPLS-DA score plot showing clear separation of pulp and seed samples based on sugar composition. (C) OPLS-DA permutation test validating model reliability ($R^2Y = 1.00$; $Q^2 = 0.988$; $p < 0.01$). (D) OPLS-DA VIP scores identifying key discriminant sugars contributing to group differentiation. (E) Heat-map clustering showing relative abundance of individual sugars across genotypes and tissues (red = high, blue = low).

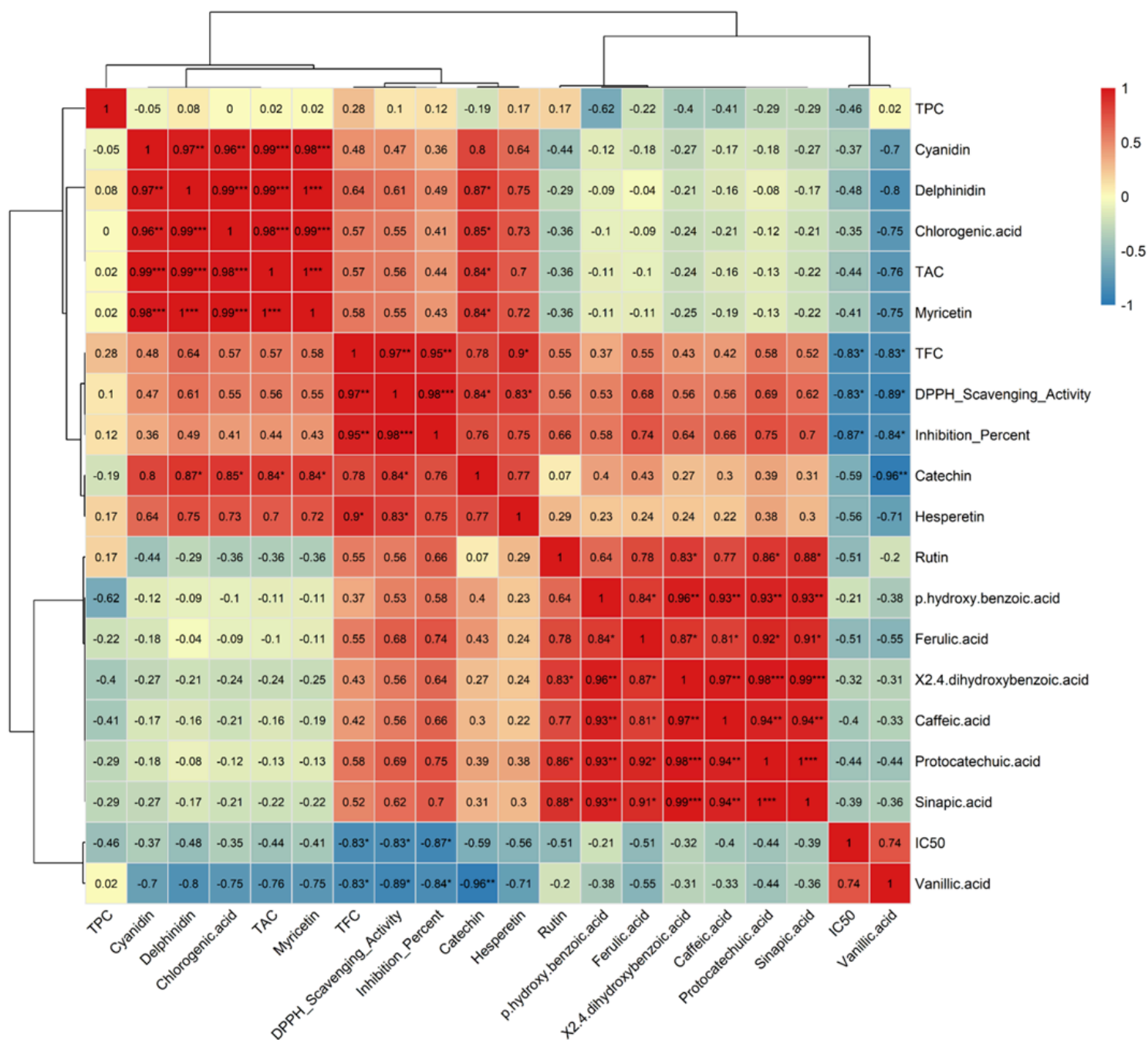


Figure 5

Heatmap of Pearson correlation coefficients (r) among phytochemicals and bioactivities. The color scale represents the strength and direction of the correlations, where red indicates a positive correlation and blue indicates a negative correlation. Variables were hierarchically clustered. Statistical significance is indicated by asterisks: *p < 0.05, **p < 0.01, and ***p < 0.001.

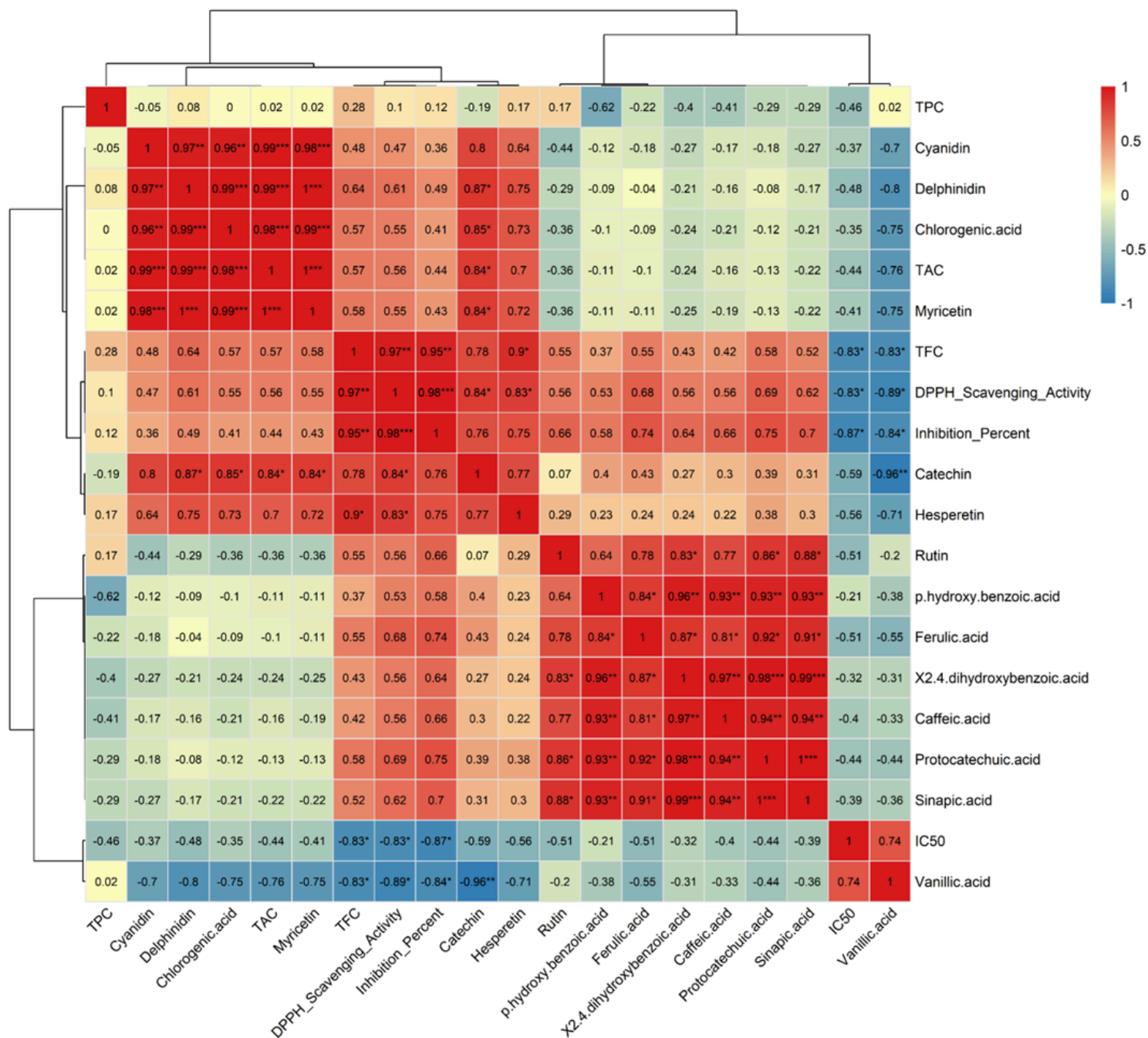


Figure 5

Heatmap of Pearson correlation coefficients (r) among phytochemicals and bioactivities. The color scale represents the strength and direction of the correlations, where red indicates a positive correlation and blue indicates a negative correlation. Variables were hierarchically clustered. Statistical significance is indicated by asterisks: *p < 0.05, **p < 0.01, and ***p < 0.001.



Figure 6

Metabolism pathways were adapted from the KEGG database (<http://www.kegg.jp/kegg/kegg1.html>) with permission from Kanehisa Laboratories. KEGG pathway metabolite interaction networks of jamun metabolites related to secondary metabolite biosynthesis (**a**) and carbohydrate metabolism (**b**). Circular nodes - KEGG pathways and square nodes - identified metabolites. Edges indicate the association between metabolites and their corresponding metabolic pathways. Edges indicate the association between metabolites and their corresponding metabolic pathways.

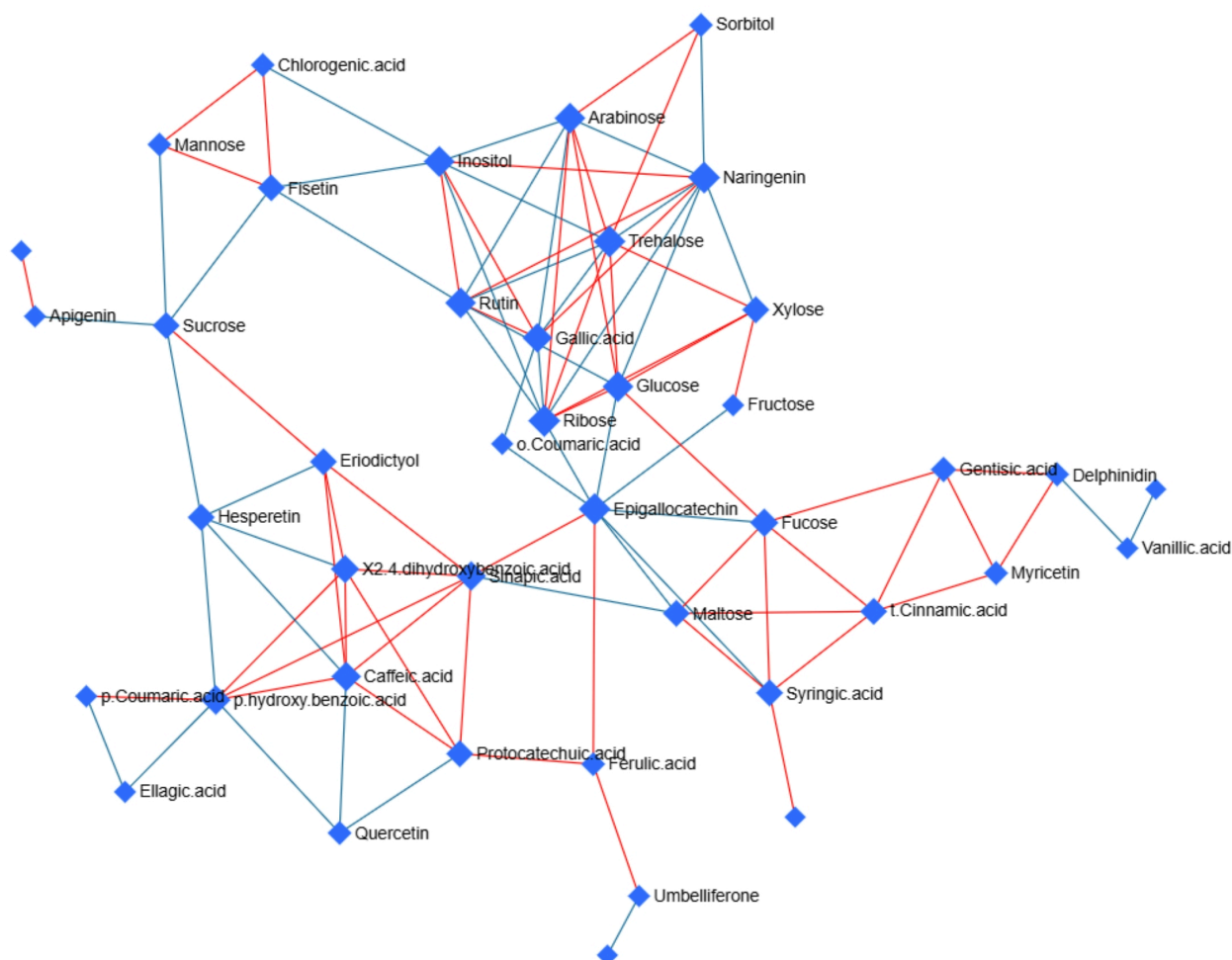


Figure 7

Network analysis of metabolite interactions in jamun *Syzygium cumini* (L.) Skeels genotypes. Correlation network displaying metabolite–metabolite associations ($r > 0.70$). Red edges represent positive correlations, while blue edges denote negative correlations. The network highlights strong clustering among phenolic acids and flavonoids forming the metabolic core, whereas sugars exhibit negative associations, reflecting contrasting primary and secondary metabolic regulation.

Supplementary Files

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- [Supplementaryfiles.docx](#)