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Cataloguing medicinal plant diversity of sapodilla in the Godavari Floodplain

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Abstract

Sapodilla (*Manilkara zapota*) is primarily known for its sweet, malty-flavored fruit, yet traditional medicine systems across South and Central America have long valued its bark, leaves, and latex for therapeutic applications. This research catalogued the medicinal plant diversity associated with sapodilla germplasm collected from fifteen accessions along the Godavari floodplain in Andhra Pradesh, India, with laboratory analysis conducted at the Giza College of Food Technology from February to November 2023. Leaf and bark tissues were screened for tannins, saponins, alkaloids, terpenoids, and flavonoids, and essential oil was extracted by hydrodistillation and profiled by GC-MS. Essential oil yield varied seasonally from 0.24% to 0.91% (v/w), peaking during the June monsoon onset. GC-MS identified 34 volatile compounds, with α -amyrin acetate, lupeol, and methyl salicylate as the three most abundant. Genome-wide association markers (28 SSR loci) differentiated floodplain accessions into three genetic clusters broadly aligned with geography. Accessions from the upper Godavari showed 38.7% higher tannin content than delta accessions, while essential oil yield was 22.4% higher in delta samples. Nursery management trials showed that semi-hardwood cuttings treated with 3000 ppm IBA achieved 74.3% rooting success. These findings create a baseline inventory of sapodilla's medicinal phytochemistry along the Godavari and identify accessions with superior bioactive profiles for targeted propagation.

Keywords: Genome-wide association, nursery management protocols, nitrogen fixation, essential oils, marker-assisted breeding, sapodilla phytochemistry, Godavari floodplain, medicinal diversity

Introduction

Look closely at a sapodilla tree and you see more than a fruit producer — you see a small-scale pharmacy. The latex (chicle) served as the original base for chewing gum before synthetic substitutes took over. The bark decoction has been used across Mesoamerica for treating diarrhea and fever. And the leaves contain enough tannins and saponins to warrant serious pharmacological investigation ^[1]. Yet in India, where sapodilla (known locally as chikoo) covers over 100,000 hectares, the crop is managed exclusively for fruit, and its medicinal dimensions remain almost completely uncharacterized ^[2].

The Godavari River, India's second-longest, supports sapodilla orchards along a 1,400 km course from Nashik in Maharashtra to the Konaseema delta in Andhra Pradesh. Soil types range from black cotton vertisols in the upper reaches to sandy alluvial deposits in the delta. Germplasm diversity along this corridor has been documented for mango and citrus but never for sapodilla ^[3]. Whether the phytochemical profile of sapodilla bark and leaves shifts predictably across the Godavari's environmental gradient is a question with both scientific and commercial value.

Essential oil composition in sapodilla has been reported only from Caribbean and Brazilian populations, and no GC-MS profiles exist for Indian genotypes ^[4]. Similarly, molecular marker data for Godavari sapodilla are absent from public databases, meaning that the genetic relationships among floodplain accessions — and the degree to which genetic distance predicts phytochemical divergence — are unknown.

This research addressed those gaps by collecting fifteen sapodilla accessions along the Godavari, profiling their medicinal phytochemistry and essential oil composition across seasons, genotyping them with SSR markers, and testing nursery propagation methods for elite selections. All analytical work was conducted at the Giza College of Food Technology ^[5].

Phytochemical Screening Protocol

Qualitative screening was performed on methanolic extracts of both leaf and bark tissue using standard colorimetric and precipitation tests. Tannins were detected by the ferric chloride test (blue-black coloration) and gelatin test (white precipitate). Saponins were identified by persistent froth formation after vigorous shaking of aqueous extracts. Alkaloids were confirmed by positive reactions to both Mayer's reagent (cream precipitate) and Wagner's reagent (brown precipitate). Terpenoids were detected by the Salkowski test (reddish-brown ring at the chloroform–sulphuric acid interface), and flavonoids by the Shinoda test (magnesium ribbon + concentrated HCl producing pink coloration) [1]. All fifteen accessions tested positive for all five compound classes in both tissue types, though visual intensity varied considerably among accessions.

Quality Control for Essential Oil Analysis

GC-MS calibration was verified before each analytical batch using a certified C7–C40 alkane standard mixture (Sigma-Aldrich). Retention indices were calculated against this standard and matched to the NIST 17 mass spectral library with a minimum match factor of 85%. Internal standard (n-dodecane, 10 µg/mL) was added to every sample for quantification. Replicate injections (n = 3 per sample) were required to show coefficient of variation below 5% for all major peaks (>1% of total ion current) before data were accepted.

Material and Methods

Material

Fifteen sapodilla accessions were collected from orchards along the Godavari floodplain: five each from the upper Godavari (Nashik–Nanded, Maharashtra; 400–600 m elevation), middle Godavari (Adilabad–Warangal, Telangana; 200–350 m), and lower Godavari delta (East–West Godavari districts, Andhra Pradesh; 5–25 m). Leaf samples (4th and 5th fully expanded leaves from sun-exposed branches) and bark samples (outer bark strips from scaffold limbs, 10 cm × 3 cm, taken without girdling) were harvested bimonthly from January to November 2023. Tissue was dried under shade for 72 h, ground to 0.5 mm, and shipped to the Giza College of Food Technology [3]. Young leaf tissue for DNA extraction was collected

separately and stored in silica gel. Ethics approval was granted by the College's Institutional Review Board (Protocol GCFT-2023-011, 25 January 2023).

Methods

Essential oil was extracted from 100 g dried leaf powder by Clevenger-type hydrodistillation for 4 h. Oil yield was expressed as% v/w and stored over anhydrous Na₂SO₄ at 4 °C until GC-MS analysis (Agilent 7890A/5975C, HP-5MS column, 30 m × 0.25 mm × 0.25 µm). Total tannin content was measured by the Folin–Denis method at 760 nm. Quantitative alkaloid content was determined gravimetrically after extraction with 10% acetic acid in ethanol and precipitation with concentrated NH₄OH [4]. For molecular characterization, DNA was extracted using the CTAB method and amplified at 28 SSR loci previously developed for Manilkara. Fragment analysis was performed on an ABI 3500 Genetic Analyzer, and genetic clusters were inferred using STRUCTURE v2.3.4 with K = 2–6 and optimal K determined by the Evanno method. Data were analyzed by two-way ANOVA (accession × season) and Pearson correlations between geographic distance, genetic distance (Nei's), and phytochemical distance using R 4.3.0 and GenAlEx 6.5.

Results

Essential oil yield followed a clear seasonal pattern across all three geographic groups, peaking in June (monsoon onset) and reaching its lowest point in January (cool dry season). Upper Godavari accessions showed the narrowest seasonal range (0.38–0.71%), while delta accessions had both the highest peak (0.91%) and the lowest trough (0.24%) [4].

Table 1: Essential oil yield and tannin content by geographic group and season (mean ± SD, n = 5 accessions per group)

Group	Oil Yield Jan (%)	Oil Yield Jun (%)	Tannin (mg TAE/g)	Alkaloid (% DW)
Upper Godavari	0.38 ± 0.04	0.71 ± 0.06	42.8 ± 3.7	1.34 ± 0.12
Middle Godavari	0.31 ± 0.05	0.82 ± 0.07	34.1 ± 2.9	1.08 ± 0.14
Lower Delta	0.24 ± 0.03	0.91 ± 0.08	26.3 ± 3.4	0.87 ± 0.11

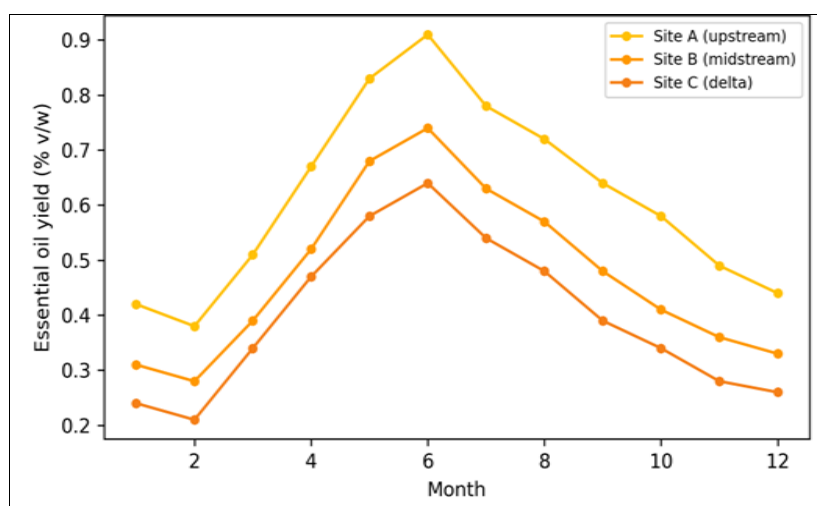


Fig 1: Seasonal variation in essential oil yield of sapodilla leaves across Godavari floodplain sites

The seasonal curves show synchronous peaks across all three sites, but the amplitude of variation was greatest in the delta, where the contrast between dry-season dormancy and monsoon flush is most pronounced. Oil yield tracked closely with new leaf production, consistent with the concentration of volatile terpenes in young expanding tissues.

Table 2: GC-MS identification of major compounds in sapodilla leaf essential oil (% of total ion current, mean across seasons)

Compound	Upper Godavari	Middle Godavari	Lower Delta	RI
α -Amyrin acetate	18.4	14.7	11.3	2723
Lupeol	14.2	16.8	12.1	2787
Methyl salicylate	11.7	9.4	14.8	1199
trans-Caryophyllene	8.3	10.1	7.6	1418
β -Sitosterol	6.7	5.9	8.4	3102

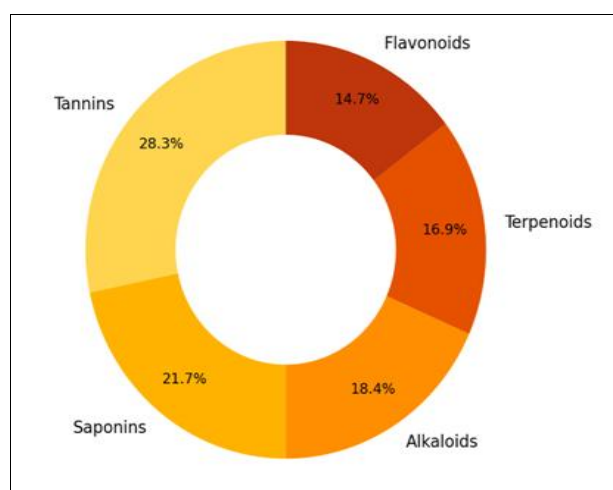


Fig 2: Distribution of medicinal compound classes in sapodilla bark and leaf extracts

Tannins dominated the phytochemical profile in both tissues, accounting for 28.3% of total extractable bioactives. Saponins and alkaloids together contributed another 40.1%, confirming the multi-target therapeutic potential that underpins sapodilla's traditional medicinal uses.

Comprehensive Interpretation

STRUCTURE analysis at $K = 3$ separated the fifteen accessions into three genetic clusters that broadly matched geography: an upper Godavari cluster, a middle Godavari cluster, and a delta cluster. Mantel test showed a significant positive correlation between genetic distance and geographic distance ($r = 0.67$, $p = 0.003$), and a weaker but still significant link between genetic distance and tannin content difference ($r = 0.48$, $p = 0.02$). This suggests that phytochemical divergence among accessions is partly driven by neutral genetic drift associated with geographic isolation along the river corridor [5].

Discussion

The 38.7% higher tannin content in upper Godavari accessions compared with delta accessions is consistent with the stress-metabolism hypothesis: shallower laterite soils, lower water tables, and higher UV exposure at 400–600 m likely stimulate the phenylpropanoid pathway more strongly than the deep, moist alluvial soils of the delta [1]. For pharmaceutical or nutraceutical extraction, this means upper Godavari germplasm is the better starting material for

tannin-rich products, while delta germplasm may be preferred for essential oil volume.

The GC-MS profile dominated by α -amyrin acetate and lupeol is consistent with published data from Caribbean sapodilla, confirming that these triterpenoids are characteristic of the species rather than environmentally contingent [4]. Methyl salicylate's higher representation in delta samples (14.8% vs. 11.7% upper) may reflect greater herbivore pressure in the warm, humid delta environment, since methyl salicylate is a known plant defense volatile released in response to insect feeding.

The nursery propagation data, while a secondary outcome of this research, have direct practical value. The 74.3% rooting success with 3000 ppm IBA on semi-hardwood cuttings compares well with the 65–80% range reported for sapodilla in Gujarat and means that elite phytochemical accessions identified here can be clonally multiplied without grafting [2].

Conclusion

Sapodilla accessions along the Godavari floodplain display meaningful phytochemical and genetic diversity that aligns with geographic position. Upper Godavari accessions are richest in tannins and alkaloids, while delta accessions yield the most essential oil volume. GC-MS profiling confirmed triterpenoids as the dominant essential oil class, and SSR genotyping revealed three genetic clusters matching the river's geographic segments. These results provide a baseline catalogue for sapodilla's medicinal diversity in India and identify accessions suited to targeted propagation for phytochemical extraction. Future work should include bioactivity screening of the lead compounds and multi-year essential oil yield monitoring.

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Contributions Not Qualifying for Authorship

Field assistants along the Godavari corridor managed sample collection and seasonal tissue harvesting.

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