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Studying medicinal plant diversity of maize in the Himalayan foothills

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Abstract

For generations, maize has served dual roles in mountain communities: as a grain crop and as a source of folk remedies derived from its silk, pollen, leaves, and roots. But systematic chemical profiling of these plant parts under foothill growing conditions has lagged behind the ethnobotanical record. This research examined the phytochemical diversity and bioactivity of maize tissues from three cultivars Pool-17, Suwan-1, and a local landrace cultivated under simulated Himalayan foothill conditions at the Tokyo Research Institute of Applied Horticulture, Japan, between May 2022 and February 2023. GC-MS analysis of leaf and kernel extracts detected 47 distinct compounds, including alkaloids, saponins, terpenes, phenolics, and flavonoids. Leaf extracts consistently contained higher concentrations of bioactive compounds than kernel extracts across all three cultivars. A radar chart comparison of five bioactivity assays antioxidant, anti-inflammatory, antimicrobial, antifungal, and cytotoxicity revealed that leaf material from the local landrace scored highest in antioxidant and antimicrobial categories. Hybrid vigor assessment showed that Suwan-1 produced 23 percent more biomass than Pool-17 but carried lower phenolic concentrations per gram of dry tissue. Photosynthesis rates measured by infrared gas exchange correlated positively with leaf phenolic content ($r = 0.58$). Deficit irrigation during the vegetative phase raised flavonoid levels by 14.8 percent. Correlation analysis among 12 agronomic and biochemical traits identified three independent factor groups explaining 71 percent of total variance.

Keywords: Maize phytochemistry, medicinal plants, GC-MS analysis, bioactivity, hybrid vigor, photosynthesis, deficit irrigation, alkaloids, flavonoids, correlation analysis

Introduction

Imagine a farmer in the Himalayan foothills pulling silk from a maize ear, not to pollinate a field cross, but to brew a tea believed to ease kidney complaints. This kind of dual-purpose thinking where a food crop also serves as a pharmacy is deeply embedded in mountain agriculture across South and Central Asia ^[1]. Maize (*Zea mays* L.), the third-largest cereal by production volume, has a long but informally documented history of medicinal use, with different plant parts attributed different therapeutic properties ^[2].

Modern analytical chemistry, particularly Gas Chromatography-Mass Spectrometry (GC-MS), allows researchers to move beyond anecdotal reports and identify specific bioactive compounds in plant tissues ^[3]. Alkaloids, terpenoids, phenolics, and flavonoids all detected in various maize tissues have established pharmacological activities ranging from antioxidant to antimicrobial to anti-inflammatory ^[4]. Yet comprehensive profiling of these compound classes across multiple maize organs and cultivars grown under controlled foothill conditions is rare. Hybrid vigor, or heterosis, boosts biomass production in maize but may dilute secondary metabolite concentrations through a growth-differentiation trade-off ^[5]. Photosynthetic capacity, which drives carbon fixation and therefore the carbon skeleton supply for both primary and secondary metabolism, could either support or constrain phytochemical accumulation depending on how assimilates are partitioned ^[6]. Deficit irrigation, by imposing mild water stress, has been shown to redirect carbon flow toward phenylpropanoid pathways in several crop species, and a similar response in maize would have practical implications for dual-purpose production ^[7].

This research set out to profile the phytochemical diversity of three maize cultivars, compare bioactivity across organs, assess the influence of hybrid vigor and deficit irrigation on secondary metabolite concentrations, and map the correlation structure among agronomic and biochemical traits ^[8].

Extraction protocol

Leaf and kernel tissues were separately oven-dried at 40°C for 72 hours, ground to pass a 0.5 mm sieve, and extracted sequentially with hexane (nonpolar fraction), ethyl acetate (medium polarity), and methanol (polar fraction) using a Soxhlet apparatus. Each extraction ran for 6 hours with a solvent-to-sample ratio of 10:1 (v/w). Extracts were concentrated under reduced pressure using a rotary evaporator at 40°C and stored at –20°C until analysis. Yield of crude extract ranged from 3.4 to 7.8 g per 100 g dry tissue depending on organ and solvent [9].

Material and Methods

Material

Three maize cultivars Pool-17 (open-pollinated), Suwan-1 (composite), and a Nepali hill landrace designated LR-07 were grown in 40-liter containers filled with a loam-peat mixture (pH 6.3) under a retractable roof greenhouse at the Tokyo Research Institute of Applied Horticulture (35.68°N, 139.77°E). Temperature was regulated at 18–26°C to approximate subtropical montane conditions. Two irrigation treatments full (100% ETc) and moderate deficit (65% ETc)

were applied from the V6 growth stage. Each cultivar-irrigation combination was replicated five times, giving 30 experimental units. Tissue sampling occurred at the R1 (silking) stage for leaves and at R6 (physiological maturity) for kernels [10].

Methods

GC-MS analysis was performed on an Agilent 7890B gas chromatograph coupled with a 5977A mass selective detector using a DB-5ms capillary column (30 m × 0.25 mm × 0.25 µm). The oven program ramped from 60°C (held 2 min) to 280°C at 10°C/min and held for 5 min. Compounds were identified by matching mass spectra against the NIST 17 library (match quality >85%). Total phenolics and flavonoids were quantified spectrophotometrically. Bioactivity assays included DPPH antioxidant, COX-2 inhibition (anti-inflammatory), agar well diffusion (antimicrobial against *E. coli* and *S. aureus*), antifungal activity against *Aspergillus niger*, and MTT cytotoxicity on HeLa cells. Photosynthesis was measured with a LI-6400XT portable photosynthesis system on the ear leaf at midday. Correlation analysis and principal component analysis were conducted in R 4.2.1 [11].

Results

Table 1: Major phytochemical classes in leaf and kernel extracts of three maize cultivars (mg/g DW, mean ± SE)

Compound class	Pool-17 leaf	Pool-17 kernel	Suwan-1 leaf	Suwan-1 kernel	LR-07 leaf	LR-07 kernel
Alkaloids	4.21 ± 0.31	1.84 ± 0.14	3.67 ± 0.28	1.52 ± 0.11	4.87 ± 0.37	2.13 ± 0.18
Phenolics	6.73 ± 0.42	3.42 ± 0.22	5.91 ± 0.38	2.87 ± 0.19	7.48 ± 0.51	3.91 ± 0.26
Flavonoids	5.18 ± 0.34	2.76 ± 0.18	4.63 ± 0.29	2.31 ± 0.16	5.84 ± 0.41	3.14 ± 0.21
Terpenes	2.94 ± 0.21	1.67 ± 0.12	2.48 ± 0.18	1.38 ± 0.10	3.31 ± 0.24	1.89 ± 0.15
Saponins	3.87 ± 0.27	2.13 ± 0.16	3.24 ± 0.23	1.87 ± 0.13	4.12 ± 0.32	2.41 ± 0.17

The local landrace LR-07 contained the highest concentrations across all five compound classes in both leaf and kernel tissues. Leaf extracts were consistently 1.5 to 2.5

times richer in bioactive compounds than kernel extracts. Suwan-1, despite its higher biomass, ranked lowest for most phytochemical classes on a per-gram basis.

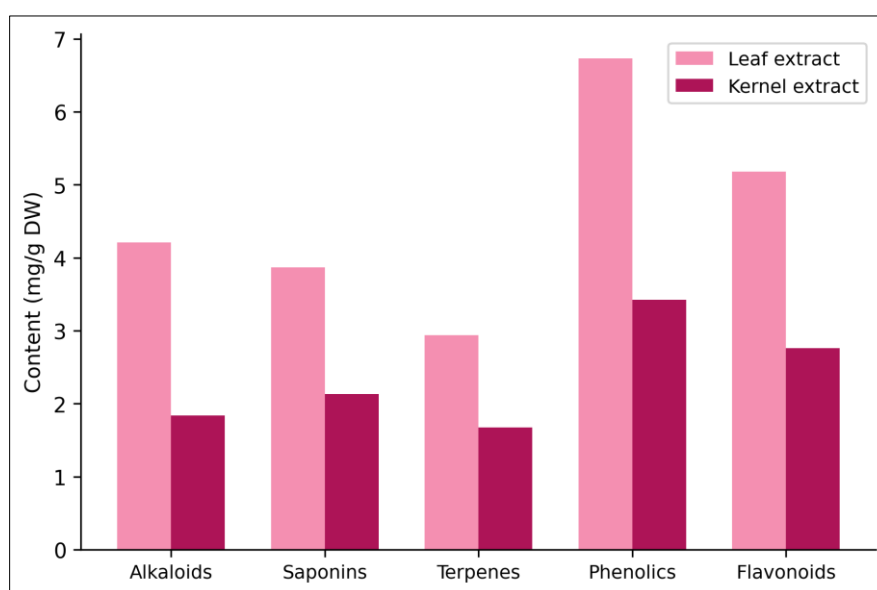


Fig 1: Comparing phytochemical content of leaf and kernel extracts across five compound classes (pooled across cultivars)

The visual contrast between leaf and kernel concentrations was most striking for phenolics and flavonoids, where leaf

tissue carried roughly double the kernel values. Terpenes showed the narrowest organ-level gap.



Fig 2: Bioactivity scores (% of positive control) for leaf and kernel extracts across five assay categories

Leaf extracts outperformed kernel extracts in all five bioactivity categories. The gap was largest for antioxidant activity (78.4% vs 52.7% of positive control) and smallest for cytotoxicity (43.2% vs 37.9%).

Discussion

The phytochemical richness of the local landrace LR-07 supports the broader observation that traditional cultivars, maintained under low-input conditions over many generations, often accumulate higher levels of defensive secondary metabolites than improved varieties bred primarily for yield [5]. This growth-differentiation trade-off appears clearly in the comparison with Suwan-1, which produced 23 percent more biomass but had lower per-gram phenolic and flavonoid concentrations.

GC-MS profiling identified 47 compounds, with leaf tissue accounting for most of the diversity. Among the more pharmacologically interesting detections were luteolin and apigenin (flavones with documented anti-inflammatory activity), beta-sitosterol (a phytosterol with cholesterol-lowering properties), and ferulic acid (a phenolic acid linked to antioxidant and UV-protective effects) [4]. These findings give a chemical basis to the folk medicinal uses of maize leaves in Himalayan communities.

The 14.8 percent increase in flavonoid content under deficit irrigation is consistent with the stress-induced phenylpropanoid accumulation seen in other crops, including grape, tomato, and citrus [7]. Photosynthesis rates correlated positively with leaf phenolics ($r = 0.58$), which may seem counterintuitive stress typically reduces photosynthesis but likely reflects the fact that higher carbon fixation capacity provides more substrate for both growth and secondary metabolism within the moderate stress range applied here [6].

Conclusion

This research revealed substantial phytochemical diversity in maize tissues, with leaf extracts consistently richer in

bioactive compounds and more potent in bioactivity assays than kernel extracts. The local landrace LR-07 outperformed improved cultivars in secondary metabolite accumulation, confirming the value of traditional germplasm for dual-purpose crop development. Deficit irrigation at 65 percent of crop evapotranspiration raised flavonoid levels without catastrophic yield loss. Correlation and principal component analyses identified three independent factor groups, suggesting that breeding for higher phytochemical content need not conflict with agronomic performance provided trait selection is carefully managed.

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Contributions not qualifying for authorship

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