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Comparative Phytochemical Profiling and Morphological Characterization of Four *Piper Betle* (Paan) Varieties Using UV–Visible Spectrophotometry

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Abstract

Piper betle L. is a widely used medicinal plant across South Asia, known for its rich phytochemical composition and significant therapeutic properties. The present investigation was conducted to comparatively evaluate four widely consumed cultivars of *Piper betle*, namely Desi paan (Sample A), Madrasi paan (Sample B), Bengali paan (Sample C), and Meetha paan (Sample D), based on their leaf morphology and phytochemical composition using UV–Visible spectrophotometric analysis. Fresh, healthy leaves devoid of any physical damage were thoroughly washed, dehydrated for 25 hours, finely powdered, and subjected to ethanolic extraction. The UV–Visible spectra recorded within the range of 200–800 nm revealed prominent absorption bands between 220–280 nm in all the studied cultivars, Reveals the existence of phenolic and flavonoid constituents. Among the four cultivars, Sample A showed the highest absorbance intensity, suggesting a comparatively higher phytochemical content, followed by Samples B and C, whereas Sample D exhibited relatively lower absorbance. Morphological assessment demonstrated notable variation in leaf size, with Desi paan possessing the largest leaves and Meetha paan the smallest. The integrated morphological and spectrophotometric results indicate that Desi paan exhibits superior phytochemical potential, highlighting its possible application in medicinal and nutraceutical formulations.

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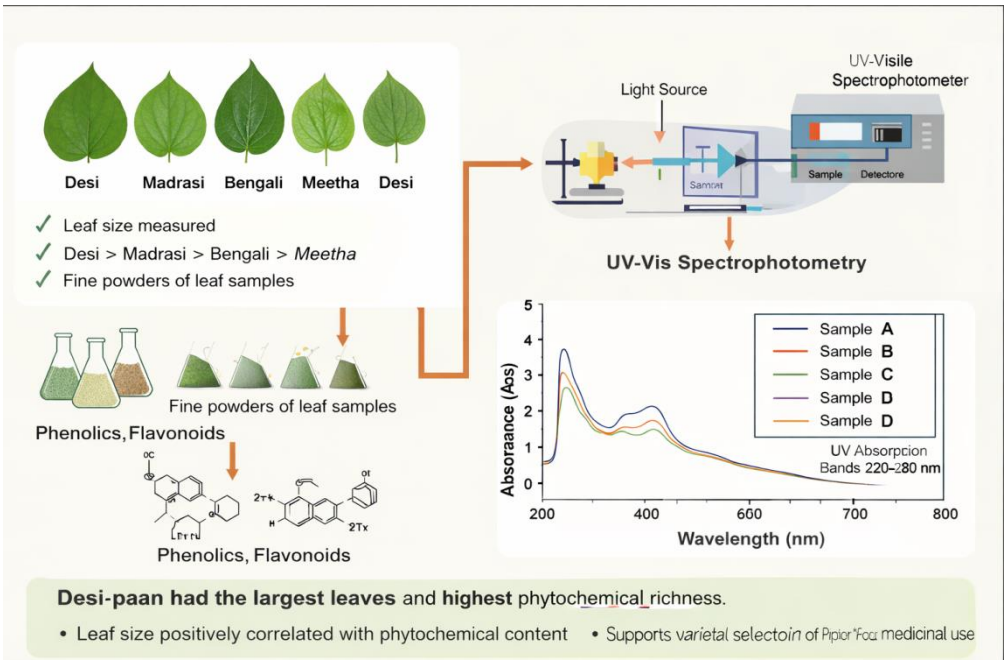


Graphical abstract; A Schematic overview illustrating the core methodology and the central finding of this study

Keywords: *Piper betl*e, UV–Visible spectrophotometry, Phytochemicals; Phenolics, Flavonoids.

1. Introduction

Piper betle L. (family Piperaceae) is an evergreen perennial climber widely cultivated and traditionally consumed across South and Southeast Asia due to its cultural, dietary, and medicinal significance. Betel leaves are well-established in indigenous medicine for their healing properties and maintain significance in both social rituals and medical applications worldwide Recently, the commercial utilization of betel leaves has expanded into food industries and pharmaceutical formulations, including confectionery products, flavored beverages, and oral hygiene products, which emphasizes the need for scientific evaluation of phytochemical variability among different cultivars.

Piper betle leaves are rich in diverse secondary metabolites like phenolics, flavonoids, terpenoids, alkaloids, and essential oils, which provide their free radical scavenging, antimicrobial, anti-inflammatory, and anticancer properties. The medicinal effectiveness of betel leaves largely depends on the qualitative and quantitative distribution of these bioactive compounds. The separation and analysis of plant biomolecules through techniques such as chromatography, centrifugation, and electrophoresis have been widely recognized as essential tools for understanding phytochemical diversity and biological activity in plants (Modi, 2024).

.Multiple research investigations have documented substantial differences in phytochemical profiles across Piper betle cultivars, attributed to genetic diversity, environmental factors, and morphological variations." Advanced analytical tools such as GC–MS and LC–MS provide detailed compound-level analysis; however, they are costly and require sophisticated instrumentation. UV–

Visible spectrophotometry serves as a rapid and cost-effective preliminary analytical method for comparative phytochemical screening, especially for phenolic and flavonoid compounds showing characteristic absorption spectra.

Morphological traits such as leaf size influence photosynthetic efficiency and metabolic activity, thereby affecting secondary metabolite accumulation. Despite this association, limited studies have correlated morphological traits with phytochemical variation in betel leaf cultivars. Therefore, the present investigation integrates morphological analysis with UV–Visible spectrophotometric profiling to comparatively evaluate four commonly consumed *Piper betle* cultivars—Desi, Madrasi, Bengali, and Meetha paan—to identify phytochemically superior cultivars for medicinal and nutraceutical applications.

MATERIALS AND METHODS

2.1 Plant Material Collection

Healthy, disease-free leaves from four Piper betle cultivars were obtained from a nearby fresh market. Leaves of consistent size, uniform color, and free from mechanical injury were chosen and designated as Sample A (Desi paan), Sample B (Madrasi paan), Sample C (Bengali paan), and Sample D (Meetha paan).

2.2 Washing, Drying, and Powder Preparation

Harvested leaves were meticulously rinsed with distilled water to remove surface dirt and microbial contaminants. Subsequently, they were dried under regulated conditions in a plant dryer for about 25 hours until fully dehydrated. The dried material was then ground to fine powder using an electric grinder and preserved in sealed containers to avoid moisture ingress and oxidative degradation. Thorough drying and pulverization are critical preliminary steps for phytochemical extraction and biochemical analyses.

2.3 Extraction Procedure

A 5 g quantity of powdered leaf sample from each cultivar underwent cold maceration extraction with 80% ethanol over 24 hours to isolate phytochemical components. The resulting extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure. Final extract volumes were standardized to ensure consistent spectrophotometric quantification

2.4 Visible Spectrophotometric Profiling

UV-Vis spectral characterization was conducted employing a double-beam spectrophotometer scanning 200-800 nm wavelength range. Pure ethanol served as blank correction. Absorbance spectra were documented and overlay graphs generated to differentiate phytochemical fingerprints across Piper betle cultivars.

2.5 Leaf Size Measurement

Leaf morphological parameters were recorded by measuring leaf length and width using a standard measuring scale. Mean values were calculated to assess morphological variation among the cultivars and to correlate leaf size with phytochemical accumulation.

Fig.; 2 Showing diff. Leaf samples of different varieties of *Piper Betel* with tools & Equipment used in Laboratory

OBSERVATIONS AND RESULTS

3.1 Morphological Characteristics

Table 1. Comparative analysis of leaf height and width (Mean ± SD) of four *Piper betle* L. cultivars. Measurements were recorded in centimeters (cm) from fresh mature leaves.

Sample	Variety	Leaf Length (cm)	Leaf Width (cm)
A	*Piper betle* L. (Desi cultivar) Desi paan	15.8 ± 0.6	10.2 ± 0.4
B	*Piper betle* L. (Madrasi culivar) Madrasi paan	14.3 ± 0.5	9.4 ± 0.3
C	*Piper betle* L. (Bengali cultivar) Bengali paan	13.6 ± 0.4	8.8 ± 0.3
D	*Piper betle* L. (Meetha cultivar) Meetha paan	12.1 ± 0.5	7.9 ± 0.4

Among the studied cultivars, Desi paan (Sample A) exhibited the highest mean leaf height and width, whereas Meetha paan (Sample D) showed comparatively smaller leaf dimensions. The observed variation in leaf morphology indicates cultivar-specific growth characteristics.

3.2 UV–Visible Spectral Analysis

Spectral analysis via double-beam UV-Vis spectrophotometer (200-800 nm) of Samples A–D (Piper betle cultivars) exhibited cultivar-specific absorbance patterns indicative of varied phytochemical profiles. Pronounced absorption maxima between 220-280 nm—typical for phenolic and flavonoid chromophores—result from $\pi \rightarrow \pi$ electronic transitions of conjugated aromatic structures. This establishes phenolic compounds as principal bioactive constituents in all analyzed betel accessions.

Sample A (Desi paan)

The UV–Visible spectrum of Sample A exhibited the highest absorbance intensity across the UV region, particularly between 220–280 nm, indicating a high concentration of phenolic and flavonoid compounds. Moderate absorbance observed in the near-UV region (300–400 nm) suggests the presence of conjugated polyphenols and tannins. A gradual decline in absorbance beyond 500 nm indicates a limited contribution of photosynthetic pigments or their reduced extractability. The broad and smooth spectral profile reflects a complex mixture of phytochemicals typical of crude plant extracts, highlighting the superior phytochemical richness and bioactive potential of this variety.

Sample B (Madrasi paan)

Sample B displayed a broad absorption pattern throughout the UV–Visible region, with a distinct absorption band between 220–280 nm, confirming the presence of phenolics and flavonoids. Moderate absorbance in the 300–400 nm range indicates conjugated aromatic compounds such as tannins and simple polyphenols. Although displaying lower overall absorbance intensity relative to Sample A, the values remained appreciable, confirming significant phytochemical load and therapeutic

Sample C (Bengali paan)

The UV–Visible spectral profile of Sample C showed clear absorbance across the entire scanned range, with a prominent UV absorption band between 220–280 nm corresponding to phenolic and flavonoid compounds. Moderate absorbance in the near-UV region indicates the presence of conjugated polyphenolic structures. The smooth absorption curve without sharp peaks suggests a complex mixture of secondary metabolites. Compared to Samples A and B, Sample C exhibited a balanced but slightly lower absorbance intensity, reflecting moderate phytochemical abundance.

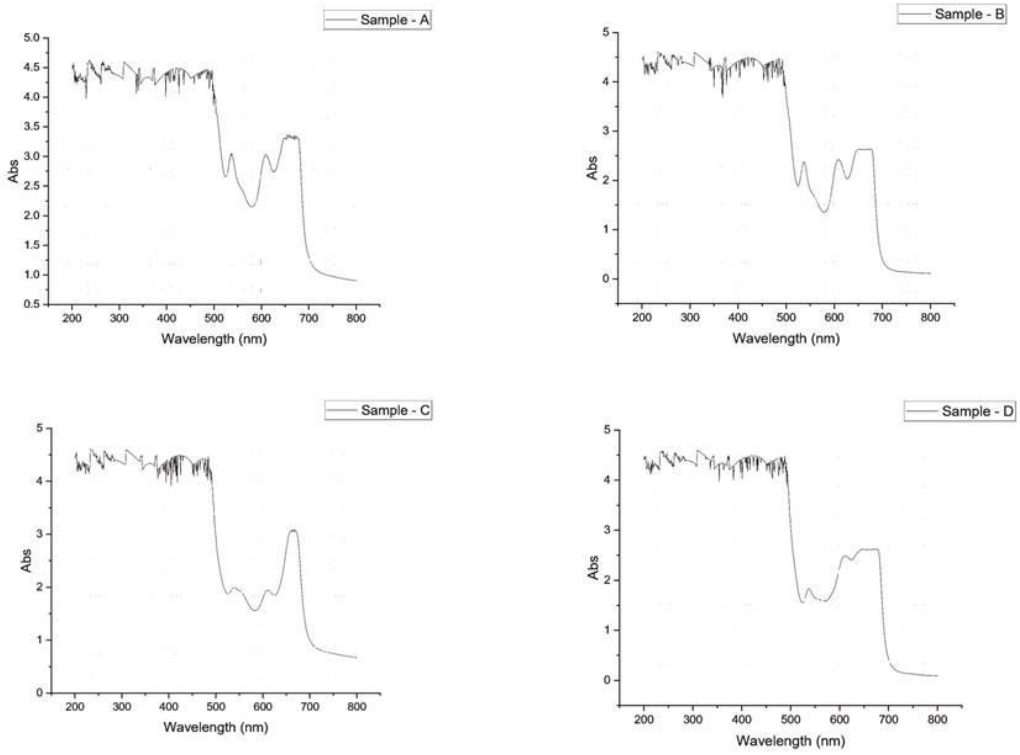
Sample D (Meetha paan)

Sample D exhibited a characteristic UV absorption band in the 220–280 nm region, confirming the presence of phenolic and flavonoid constituents. Moderate absorbance in the 300–400 nm region suggests conjugated polyphenols and related aromatic compounds. However, the overall absorbance intensity of Sample D was comparatively lower than the other varieties, indicating reduced phytochemical concentration, although the presence of bioactive secondary metabolites was clearly evident.

Comparative Interpretation

Comparative analysis of the UV–Visible spectra revealed quantitative variation in phytochemical composition among the four *Piper betel* varieties. Sample A exhibited the highest absorbance intensity, indicating superior phytochemical richness, followed by Samples B and C, while Sample D showed comparatively lower but significant phytochemical content. The consistent presence of UV absorption bands between 220–280 nm in all samples confirms phenolics and flavonoids as dominant constituents. The observed variations in absorbance intensity may be attributed to genetic differences among cultivars as well as environmental factors influencing secondary metabolite biosynthesis.

Sample	Variety Name	Average Leaf Size*	UV–Visible Absorbance Intensity	Major Phytochemical Indication	Medicinal Potential
A	Desi paan	Large	Very High	High phenolics & flavonoids	Very High
B	Madrasi paan	Medium–Large	High	Moderate to high phenolics	High
C	Bengali paan	Medium	Moderate	Moderate phenolics	Moderate
D	Meetha paan	Small	Low	Low phenolic & flavonoid content	Low



Graph-Showing Phytochemical Analysis of different varieties of Piper beetle.

DISCUSSION

The UV-Visible spectral signatures recorded in this investigation unequivocally establish the presence of phenolic and flavonoid metabolites across all four Piper betle L. cultivars. These polyphenolic compounds characteristically display intense UV absorption (220-280 nm) attributable to $\pi \rightarrow \pi$ transitions in aromatic and conjugated chromophores, thereby validating UV-Vis spectrophotometry as an efficient preliminary screening method for phytochemical fingerprinting and cultivar comparison (Harborne, 1998; Pandey & Tripathi, 2014; Skoog et al., 2014)

Among the cultivars examined, Desi paan exhibited the highest UV absorbance intensity, signifying superior accumulation of bioactive phytochemicals. Higher phenolic and flavonoid levels have been reliably linked to improved antioxidant and antimicrobial properties in Piper betel leaves (Baliga et al., 2011; Chakraborty et al., 2020; Das et al., 2019). These results offer biochemical validation for the traditional preference of Desi paan and highlight its enhanced therapeutic value, especially for combating oxidative stress and microbial infections (Cowan, 1999)

A key finding from this study is the correlation between leaf morphology and phytochemical content. Leaf size significantly affects photosynthetic capacity and metabolic efficiency, thereby influencing secondary metabolite production and accumulation. The progressive decrease in UV absorbance from Desi paan to Meetha paan paralleled diminishing leaf size, indicating a positive correlation between leaf area and phytochemical richness. Previous research has emphasized morphological traits' role in betel leaf quality and medicinal value (Guha, 2006), though experimental confirmation remains limited.

.Madrasi paan displayed intermediate UV absorbance levels, signifying moderate phytochemical accumulation—less than Desi paan but substantial. Conversely, Meetha paan recorded the lowest absorbance, corresponding to diminished phenolic and flavonoid concentrations. This aligns with prior studies indicating that cultivars bred mainly for palatable flavor and fragrance often exhibit reduced therapeutic efficacy (Kumar et al., 2010; Chakraborty et al., 2020). Thus, despite Meetha paan's popularity in cultural and culinary contexts, it proves less ideal for medicinal or phytopharmaceutical uses.

The innovative aspect of this research is the combined application of UV-Visible spectrophotometry and morphological analysis to distinguish Piper betel cultivars by their phytochemical content. In contrast to previous investigations that predominantly employed sophisticated chromatography or biochemical methods, this study proves that a straightforward, economical spectrophotometric method—paired with leaf morphology—successfully identifies high- versus low-phytochemical varieties. This strategy holds special value for quick screening in resource-constrained environments (Harborne, 1998; Pandey & Tripathi, 2014).

Overall, the findings scientifically validate traditional knowledge favoring Desi paan for medicinal use and provide a morphology-based explanation for its superior phytochemical composition. The study contributes to a better understanding of structure–function relationships in Piper betel and supports informed varietal selection for therapeutic, nutraceutical, and phytopharmaceutical applications (Baliga et al., 2011; Poudel et al., 2023).

Conclusion

This research underscores pronounced varietal differences in Piper betel cultivars in both morphological traits and phytochemical profiles among the evaluated Piper betel cultivars. Desi paan emerged as the most phytochemically enriched variety, characterized by a larger leaf area and higher accumulation of UV-absorbing bioactive compounds. These attributes indicate its superior suitability for medicinal, nutraceutical, and functional applications. The findings also demonstrate that UV–Visible spectrophotometry can serve as a reliable and economical screening method for preliminary differentiation of Piper betel varieties based on phytochemical abundance. Collectively, the results lend scientific support to traditional knowledge systems and reinforce the relevance of Desi paan as a valuable natural resource with functional and therapeutic significance.

Recommendations and Future Scope

- Desi paan should be prioritized for the development of antioxidant-rich herbal formulations and value-added products.
- Comprehensive identification and quantification of bioactive constituents using advanced techniques such as HPLC and GC–MS are warranted.
- Future investigations should examine the relationship between leaf morphological attributes and biological activities to strengthen structure–function interpretations.
- Establishing varietal standardization protocols based on morphological and phytochemical parameters will enhance the quality control of Piper betel-derived herbal and phytopharmaceutical products.

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