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Solvent-Dependent Phytochemical Profiling and Antioxidant Potential of Piper betle Leaves and Piper nigrum Seeds

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Abstract

This study analysed the phytochemical composition and antioxidant activity of Piper betle leaves and Piper nigrum seeds extracted using solvents of varying polarity: hydroethanol, ethanol–hexane, and methanol. Quantitative analysis revealed marked differences in phenolic, flavonoid, and terpene contents among the extracts. The hydroethanolic extract of P. betle (B1) exhibited the highest concentrations of phenols (787.08 ± 59.32 µg/mL) and flavonoids (576.04 ± 23.56 µg/mL), whereas the ethanol–hexane extract of P. nigrum (P2) contained the highest terpene level (1027.69 ± 38.77 µg/mL). These findings highlight the superior efficiency of polar solvents for extracting phenolic and flavonoid compounds from P. betle, while non-polar systems favoured terpene recovery from P. nigrum. Antioxidant activity, evaluated using the DPPH radical scavenging assay, demonstrated strong inhibition across all P. betle extracts (>91%), with the hydroethanolic extract (B1) achieving the highest scavenging activity (94.00%). In contrast, P. nigrum seed extracts showed substantially lower activity, with inhibition values between 24.22% and 24.44%, irrespective of solvent type. Overall, the comparative analysis establishes P. betle leaves as a richer source of phenolic and flavonoid constituents with robust antioxidant potential, while P. nigrum seeds predominantly contribute terpene compounds with comparatively limited radical scavenging activity. These results underscore the greater potential of P. betle for applications in functional foods, nutraceuticals, and therapeutic formulations.

Keywords: Herbal extracts, solvent extraction, phytochemical, antioxidant, DPPH assay.

1. Introduction

Historically, herbs and spices have been valued for their dual role in enhancing food flavour and preservation, as well as for their medicinal benefits (Nagalingam and Arumugam, 2011). Traditional medical systems continue to rely heavily on medicinal plants, largely due to their extensive pharmacological efficacy and minimal side effects in biological systems (Damanhouri and Ahmad, 2014). The Piper betle plant, from the Piperaceae family, is a perennial and evergreen climber notable for its glossy, cordate leaves and white catkins. It has been traditionally used in South and Southeast Asia, including India, China, and Thailand. The leaves, roots, stems, stalks, and fruits of P. betle are valuable components used in traditional medicine. These leaves produce an aromatic, light-yellow essential oil, notable for its sharp,

burning taste and attributed bioactive properties (Santhakumari et al., 2003). The leaves of Piper betle serve as a reservoir for numerous bioactive molecules, notably polyphenols, alkaloids, flavonoids, terpenes, steroids, saponins, and tannins, all of which exhibit various biological activities (Adhikary et al., 1998). By neutralising free radicals, Phytochemical-rich dietary substances with antioxidant properties help to prevent or delay the oxidative degradation of lipids, proteins, and nucleic acids, which is implicated in the pathogenesis of various chronic diseases (Lim et al., 2007). Numerous studies have shown that antioxidants of P. betle can decrease the risk of death from cardiovascular diseases and contribute to the prevention of cancer and other long-term illnesses (Anani et al., 2000; Devasagayam et al., 2004; Agoramoorthy et al., 2008).

The spice Piper nigrum L. (Family: Piperaceae), known as 'The King of Spices', contains piperine, a major alkaloid with various therapeutic potentials. Piperine, the principal alkaloid of Piper nigrum L., enhances the bioavailability of drugs and nutrients by inhibiting metabolic enzymes. It also demonstrates a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antitumor, antimicrobial, hepatoprotective, and neuroprotective effects, among others (Damanhouri and Ahmad, 2014). This herb has been proven to improving neurological function and support reproductive capacity (Wattanathorn, et al., 2008). Black pepper is a widely valued spice, known for its distinctive aroma and pungent flavour (El-Mofty et al; 1988). The antioxidants present in this plant serve as essential additives in the food industry to delay or prevent oxidative damage (Gülçin et al., 2005). Antioxidant therapies have shown potential in preventing and managing multifactorial diseases such as atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer (Devasagayam et al., 2004).

2. Materials and methods

2.1. Collection of herbal plants

The leaves of the P. betle plant originated from Athoor, Thoothukudi, Tamilnadu which are traditionally called as Athoor betel leaves. These leaves were collected from the Botanical Garden, Nagercoil, Tamilnadu. The seeds of the P. nigrum plant were the origin of Pathanamthitta, Kerala, which is also a traditional species, was collected from the pepper estate of Paricode, Nagercoil, Tamilnadu. The leaves and seeds were washed thoroughly in distilled water and shade-dried to remove the moisture content of the leaves and seeds. After complete drying, the leaves and seeds were finely powdered and sieved through a mesh and stored in air-tight containers for future use.

Figure 2.1. A. Piper betle (Betel plant) **B. Piper nigrum (Black pepper plant)**

2.2. Preparation of herbal plant extracts

The plant extracts were obtained using the Soxhlet extraction method, as described by Wang and Weller, 2006. For each sample, 15 grams of dried, powdered plant material were loaded into a thimble and subjected to extraction with 150 ml of solvent. The Soxhlet apparatus was set up, and the process was conducted for 6 hours at the boiling point specific to each solvent used. Following extraction, the solvents were removed under reduced pressure using a rotary evaporator to yield the concentrated crude extract. These extracts were then stored at 4 °C until further analysis. Various solvents—water, ethanol, hexane, and methanol—were utilized either individually or in combination, as outlined in Table 2.2.

Table 2.2. Sample Codes and Solvent Systems for Herbal Extracts

Sample Code	Sample Description
B1	Piper betle leaves extract with 30% water & 70% ethanol mixed solvent
B2	Piper betle leaves extract with 3 0% ethanol & 70% hexane mixed solvent
B3	Piper betle leaves extract with 100% methanol
P1	Piper nigrum seeds extract with 30% water & 70% ethanol mixed solvent
P2	Piper nigrum seeds extract with 30% ethanol & 70% hexane mixed solvent
P3	Piper nigrum seeds extract with 100% methanol

2.3. Quantitative phytochemical analysis of herbal plant extracts

The herbal extracts derived from P. betle leaves and P. nigrum seeds were analysed quantitatively to determine the levels of key bioactive constituents, including phenols, flavonoids, and terpenes (Egbuna et al., 2018).

2.4. Antioxidant activity of herbal plant extracts by DPPH Assay

The prepared herbal extracts of P. betle leaves and P. nigrum seeds were subjected to antioxidant activity to find the inhibition percentage of antioxidants. The antioxidant activity was tested by the DPPH assay method (Zhang and Xu, 2015).

3. Result and discussion

3.1. Quantitative Phytochemical Analysis

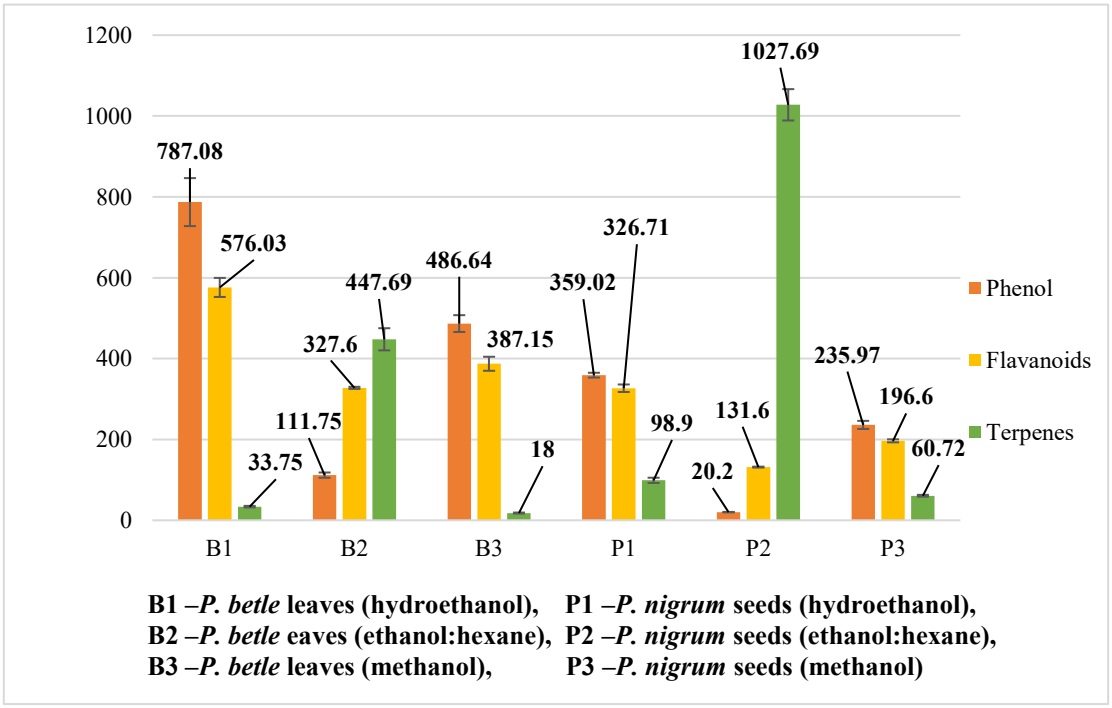
The quantitative evaluation of phytochemicals in Piper betle leaves and Piper nigrum seeds extracted with various solvent systems demonstrated notable variations in the concentrations of phenols, flavonoids, and terpenes (Figure 3.1 and Table 3.2). Among the tested samples, the hydroethanolic extract of P. betle (B1) recorded the highest phenolic content at 787.08 ± 59.32 µg/ml, affirming the effectiveness of polar solvents in dissolving phenolic compounds. Methanolic extract B3 and hydroethanolic extract P1 also showed substantial phenol levels at 486.64 ± 20.76 µg/ml and 359.02 ± 5.95 µg/ml, respectively. The methanolic extract of P. nigrum (P3) contained moderate levels at 235.97 ± 10.0 µg/ml. A significant reduction in phenolic concentration was observed in B2 (111.75 ± 6.51 µg/ml), while P2 (ethanol: hexane extract) had the lowest phenolic content at 20.20 ± 0.57 µg/ml. These findings indicate that P. betle generally yields a higher phenolic content than P. nigrum, and that polar solvents like hydroethanol and methanol are more effective for extracting phenols.

Regarding flavonoids, the highest amount was again observed in the hydroethanol extract of P. betle (B1) at 576.04 ± 23.56 µg/ml, demonstrating the solvent's efficiency for flavonoid extraction. This was followed by the methanol extract (B3) at 387.15 ± 17.36 µg/ml and the ethanol: hexane extract (B2) at 327.60 ± 2.55 µg/ml. In the case of P. nigrum, the hydroethanol (P1) and methanol (P3) extracts yielded 326.71 ± 9.26 µg/ml and 196.60 ± 3.75 µg/ml, respectively. The lowest flavonoid content was measured in P2 (ethanol: hexane) at 131.60 ± 1.13 µg/ml. Overall, P. betle particularly, the B1 extract, demonstrated higher flavonoid levels than P. nigrum, and polar solvents outperformed non-polar combinations in flavonoid extraction.

In contrast to the patterns observed for phenols and flavonoids, the ethanol: hexane extract of P. nigrum (P2) exhibited the highest terpene content at 1027.69 ± 38.77 µg/ml, indicating that non-polar solvents are more efficient in extracting terpenes. B2 (ethanol: hexane extract of P. betle) followed with 447.69 ± 27.42 µg/ml, while P1 (hydroethanol extract of P. nigrum) showed 98.90 ± 6.47 µg/ml. Minimum terpene concentrations were observed in P3 (60.72 ± 2.33 µg/ml) and B1 (33.75 ± 1.93 µg/ml). The lower terpene content was found in the methanol extract of P. betle (B3) at 18.00 ± 1.18 µg/ml. These observations suggest that P. nigrum, particularly when extracted with non-polar solvents, is a richer source of terpenes compared to P. betle, and that solvent polarity plays a significant role in terpene yield.

These outcomes are in agreement with previous research. For instance, methanolic extracts of P. betle leaves have been reported to contain high levels of phenols, flavonoids, and tannins (Basit et al., 2023; Bratati et al., 2022). Similarly, P. nigrum seed extracts in methanol have shown notable amounts of phenols and flavonoids (Ahmad et al., 2015). Hydroalcoholic extracts of P. nigrum seeds have been found to contain higher concentrations of these compounds than their aqueous counterparts (Zhao et al., 2024).

Figure 3.1. Quantitative phytochemical analysis of P. betle leaves and P. nigrum seeds Extracts



B1 –*P. betle* leaves (hydroethanol), P1 –*P. nigrum* seeds (hydroethanol),
B2 –*P. betle* eaves (ethanol:hexane), P2 –*P. nigrum* seeds (ethanol:hexane),
B3 –*P. betle* leaves (methanol), P3 –*P. nigrum* seeds (methanol)

All values are expressed as mean ± standard error (SE) of three replicates (n = 3).

3.2. Antioxidant Activity of herbal extracts (DPPH Assay)

The antioxidant potential of herbal extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The absorbance of the DPPH control solution (blank) was recorded at 0.900 at 517 nm**,** and the percentage inhibition for each sample was calculated across a range of concentrations from which the maximum inhibition values were derived. The six test samples included extracts from Piper betle leaves and Piper nigrum seeds, extracted using different solvent systems: hydroethanol, ethanol: hexane and methanol (Table 3.1).

Table 3.2. Antioxidant Activity of herbal plant extracts as measured by DPPH radical Scavenging

Sample	Plant Source	Solvent Extract Type	Maximum % Inhibition	Antioxidant Potential
B1	P. betle leaves	Hydroethanol	94.00%	Very High
B2	P. betle leaves	Ethanol: Hexane	91.78%	Very High
B3	P. betle leaves	Methanol	93.44%	Very High
P1	P. nigrum seeds	Hydroethanol	24.22%	Low
P2	P. nigrum seeds	Ethanol: Hexane	24.44%	Low
P3	P. nigrum seeds	Methanol	24.22%	Low

The results clearly demonstrate a distinct contrast in antioxidant capacity between the P. betle leaf extracts and the P. nigrum seed extracts. All three P. betle extracts exhibited excellent DPPH radical scavenging activity, with percentage inhibitions consistently exceeding 91%. The highest inhibition was observed in B1 (hydroethanol extract) at 94.00%**,** suggesting a strong presence of polar antioxidant compounds. This was closely followed by B3 (methanol extract) at 93.44% and B2 (ethanol: hexane extract) at 91.78%**.**

These high values indicate the effectiveness of P. betle leaves as a source of potent antioxidant compounds. The consistency across solvent systems suggests that the antioxidant molecules in P. betle are likely to be both polar and semi-polar in nature, which are effectively extracted across different solvent polarities. In contrast, all P. nigrum seed extracts exhibited much lower antioxidant potential, with maximum inhibition values ranging from 24.22% to 24.44%**.** The similarity across P1, P2, and P3 suggests that solvent choice did not significantly influence the extraction of antioxidant compounds from P. nigrum seeds. This indicates a lower inherent antioxidant compound content in the seeds. The result of this study was concurrent to the earlier research in that, the ethyl acetate and ethanolic extract of P. betle leaves exhibited higher inhibition percentage of DPPH scavenging activity (Risdian et al., 2011). The P. betle leaves extracted with methanol and ethyl acetate showed higher DPPH scavenging activity when compared with the P. betle leaves hexane extract (Mokhtar et al., 2008). The aqueous extract of P. nigrum seeds showed lower-level DPPH scavenging activity when compared with other extracts (Glucin, 2005). The ethanolic extracts of P. nigrum seeds revealed higher DPPH scavenging activity than the aqueous extracts of P. nigrum seeds (Nahak and Sahu, 2011).

4. Conclusion

This study highlights the significant influence of solvent polarity on the extraction efficiency of bioactive compounds from Piper betle leaves and Piper nigrum seeds. Polar solvents, particularly hydroethanol and methanol, facilitated higher recovery of phenols and flavonoids, especially in P. betle, which also exhibited superior antioxidant activity across all extracts. In contrast, non-polar solvents favoured the extraction of terpenes, particularly from P. nigrum. These findings underscore the importance of solvent selection in optimizing phytochemical yield and antioxidant potential, with P. betle emerging as a promising source of natural antioxidants.

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