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Effect of Solvent Polarity on the Selective Extraction of Bioactive Compounds from *Piper betle* Leaves and *Piper nigrum* Seeds

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Abstract
A comparative investigation was conducted to evaluate the phytochemical composition of *Piper betle* (betel) leaves and *Piper nigrum* (black pepper) seeds using solvents of varying polarity. Soxhlet extraction was performed with three solvent systems: hydroethanol (30% water: 70% ethanol), ethanol: hexane (30% ethanol: 70% hexane), and absolute methanol. The extracts were subjected to both qualitative phytochemical screening and quantitative estimation of total phenolic content (using the Folin–Ciocalteu method), flavonoid content (via the aluminium chloride assay), and terpene content (using the vanillin–sulphuric acid method). The qualitative analysis revealed the presence of phenols, flavonoids, and terpenes in all *P. betle* leaf extracts, regardless of the solvent system. In the case of *P. nigrum* seed extracts, hydroethanol and methanol successfully extracted all three phytochemicals, whereas the ethanol: hexane extract lacked detectable phenolic compounds. Quantitative results indicated that the hydroethanolic extract of *P. betle* (B1) exhibited the highest phenolic (787.08 ± 59.32 µg/mL) and flavonoid (576.04 ± 23.56 µg/mL) content. Conversely, the ethanol: hexane extract of *P. nigrum* yielded the highest terpene concentration (1027.69 ± 38.77 µg/mL).Overall, the study underscores the influence of both plant species and solvent polarity on phytochemical extraction efficiency. *Piper betle* leaf extracts, particularly those obtained with hydroethanol, were notably rich in phenolic and flavonoid compounds, while *Piper nigrum* seed extracts with ethanol: hexane exhibited enhanced terpene content.

Keywords: Solvent polarity, phytochemicals, phenolics, flavonoids, terpenes, Soxhlet extraction

1. Introduction

Herbs and spices have long been valued not only for their roles in enhancing flavour and preserving food but also for their therapeutic benefits (Nagalingam and Arumugam, 2011). Traditional medicinal systems, such as Ayurveda, Homoeopathy, Unani, and Siddha, rely extensively on plant-derived remedies, with approximately 90–95% of formulations being plant-based. In recent decades, there has been a resurgence of interest in ethnomedicinal plants due to their cultural acceptance, biocompatibility, and reduced side effects compared to synthetic pharmaceuticals (Kumar et al., 2010; Savithramma, 2011).

Piper betle is commonly known as betel leaf; the genus Piper belongs to the family Piperaceae. It is a perennial evergreen vine, holds significant cultural and medicinal value across Southeast Asia. Traditionally revered in many cultures, its leaf shape is often symbolically linked to the human heart (Pradhan, 2013). Widely cultivated in humid tropical regions, *P. betle* has been used in various traditional practices due to its therapeutic potential. The leaves are known to enhance digestive functions, stimulate pancreatic lipase activity, and are used in treating ailments such as respiratory conditions, abscesses, conjunctivitis, and constipation (Prabhu et al., 1995). In Ayurvedic medicine, *P. betle* is believed to support cardiovascular function, balance the vata and kapha dosha, and help clear mucus from the respiratory tract due to its heating nature (Balkrishna, 2008). Its pharmacological potential is attributed to a wide spectrum of bioactive compounds, including phenolics, flavonoids, and terpenes, distributed throughout the plant (Mohanto et al., 2017). Several studies have documented the biological activities of *P. betle*, including antimicrobial, antifungal, antioxidant, antidiabetic, gastroprotective, cytotoxic, anti-inflammatory, and anticancer properties (Pawar et al., 2021).

Piper nigrum is commonly known as black pepper. The genus Piper belongs to the family Piperaceae, and represents one of the most ancient lineages of flowering plants, distributed in the tropical regions, and is well known for its ecological diversity and long- standing ethno botanical importance (Scott *et al*., 2007). It is widely recognised for its culinary use and is also referred to as the "King of Spices." The plant has drawn considerable attention in both traditional and modern medicine for its therapeutic potential (Takooreea et al., 2019). In traditional healthcare systems, *P. nigrum* has been employed to treat conditions such as colds, digestive disorders, respiratory ailments, and inflammatory diseases (Ahmad et al., 2012; Szallasi, 2005). India, in particular, records the highest number of traditional medicinal uses of *P. nigrum*, where its seeds and fruits are processed into powders, pills, tablets, or pastes for the treatment of gastrointestinal, respiratory, and reproductive conditions (Takooreea et al., 2019). The plant's bioactive constituents have demonstrated significant pharmacological effects, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and

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anticancer activities (Ahmad et al., 2012; Damanhouri and Ahmad, 2014). *P. nigrum* extracts have also shown efficacy against multidrug-resistant pathogens through mechanisms such as biofilm disruption, inhibition of bacterial efflux pumps, and interference with motility (Takooreea et al., 2019). Its seeds are rich in secondary metabolites, including phenolic acids, flavonoids, and terpenoids, which are key contributors to its medicinal potential (Damanhouri and Ahmad, 2014).

In the present study, a comparative qualitative and quantitative analysis was carried out to evaluate the phytochemical content of *Piper betle* leaves and *Piper nigrum* seeds extracted using solvents of varying polarity. This research aims to assess how solvent composition influences the extraction efficiency of key phytochemicals such as phenols, flavonoids, and terpenes from these medicinally important plants.

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 and it 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 leaves. After complete drying the leaves and seeds were finely powdered and sieved through a mesh and stored in air-tight containers for future use. The systematic classification of the selected herbal plants is given in Table 2.1.

Table 2.1. Systematic classification of selected herbal plants

S. No	Botanical name	Common name	Kingdom	Phylum	Class	Order	Family
1	*Piper betle*	Betel	Plantae	Magnoliophyta	Magnoliopsida	Piperales	Piperaceae
2	*Piper nigrum*	Black Pepper	Plantae	Tracheophyta	Magnoliopsida	Piperales	Piperaceae

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 prepared using the Soxhlet extraction technique (Wang and Weller, 2006). For each extraction, 15 grams of dried and powdered plant material were placed in a thimble and extracted using 150 mL of solvent. The Soxhlet apparatus was assembled, and the extraction was carried out for 6 hours at the boiling point of the respective solvent system. After extraction, the solvent was evaporated under reduced pressure using a rotary evaporator to obtain the concentrated crude extract, which was then stored at 4°C until further use. Different solvents, including water, ethanol, hexane, and methanol, were used either alone or in combination, as detailed 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 30% 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

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2.3. Qualitative phytochemical analysis of herbal plant extracts
The prepared herbal extracts of *P. betle* leaves and *P. nigrum* were subjected to qualitative phytochemical analysis to determine the presence of important bioactive components such as phenols, flavonoids and terpenes (Harbone J.B, 1998).

2.3.1. Test for phenols
To the extract, 2 ml of distilled water and a few drops of 10 % ferric chloride were added. The formation of blue or green colour shows the presence of phenols.

2.3.2. Test for flavonoids
A few drops of diluted sodium hydroxide were mixed into 1 ml of extract. Yellow colour indicates the presence of flavonoids.

2.3.3. Test for terpenoids
2 ml of chloroform was added to 5ml of the extract, and concentrated sulphuric acid was added to form a layer. A reddish-brown colour appears which indicates the presence of terpenoids.

2.4. Quantitative phytochemical analysis of herbal plant extracts
The prepared herbal extracts of *P. betle* leaves and *P. nigrum* seeds were subjected to quantitative phytochemical analysis to quantify the important bioactive components such as phenols, flavonoids and terpenes present in these extracts (Egbuna *et al.,* 2018).

2.4.1. Quantitative analysis of total phenols (Folin-Ciocalteu method)
The total phenolic content (TPC) of the extracts was determined using the Folin–Ciocalteu method. In this procedure, 0.5 mL of the plant extract (appropriately diluted in methanol) was mixed with 2.5 ml of diluted Folin–Ciocalteu reagent. After 5 minutes of incubation at room temperature, 2.0 ml of 7.5% sodium carbonate solution was added to the reaction mixture. The mixture was vortexed and incubated at room temperature in the dark for 30 minutes. The absorbance of the blue-coloured complex was measured at 765 nm using a UV–VIS spectrophotometer. A calibration curve was prepared using Gallic acid at different concentrations (10–100 µg/mL), and the total phenolic content was expressed as mg Gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract).

2.4.2. Quantitative analysis of total flavonoids (aluminium chloride method)
The total flavonoid content (TFC) of the herbal extracts was estimated using the aluminium chloride method. In this procedure, 1 mL of the plant extract (appropriately diluted in ethanol) was mixed with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. After 5 minutes, 0.3 mL of a 10 % aluminium chloride solution was added, followed by incubation for 6 minutes at room temperature. Then, 2 mL of 1 M sodium hydroxide was added to the mixture, and the total volume was made up to 10 mL with distilled water. The reaction mixture was mixed thoroughly, and the absorbance was measured at 510 nm using a UV–Vis spectrophotometer. Quercetin was used to prepare the calibration curve, and the results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

2.4.3. Quantitative analysis of total terpenes (vanillin-sulphuric acid method)
The total terpene content (TTC) of the herbal extracts was determined using the vanillin–sulphuric acid method. In this assay, 1 ml of the plant extract (diluted in methanol) was mixed with 2.5 ml of vanillin reagent (5% vanillin in glacial acetic acid) and 2.5 ml of concentrated sulphuric acid. The mixture was incubated at 60°C for 15 minutes in a water bath and then cooled to room temperature. The absorbance of the resulting reddish-pink colour was measured at 538 nm using a UV–VIS spectrophotometer. Linalool terpene standard was used to generate the calibration curve, and the total terpene content was expressed as milligrams of linalool equivalents per gram of extract (mg LE/g extract).

3. Result and discussion
3.1. Qualitative phytochemical analysis
The qualitative phytochemical screening of *Piper betle* leaves and *Piper nigrum* seeds extracted using different solvent systems confirmed the presence of key secondary metabolites—phenols, flavonoids, and terpenoids (Table 3.1). All extracts of *P. betle* (B1 – hydroethanol, B2 – ethanol: hexane, and B3 – methanol) tested positive for phenolic compounds, indicating good solubility of phenols in both polar and mixed solvent systems. These findings are consistent with previous reports where methanolic extracts of *P. betle* leaves were shown to contain phenols, flavonoids, terpenoids, and other phytochemicals (Basit *et al*., 2023). Similarly, (Saini et al., 2016) reported the presence of phenols and flavonoids in the ethanolic extract of *P. betle* leaves.
In contrast, the ethanol: hexane extract of *P. nigrum* seeds (P2) showed an absence of phenolic compounds, while hydroethanol (P1) and methanol (P3) extracts confirmed their presence. This indicates a reduced solubility or affinity of phenolic compounds from *P. nigrum* in non-polar solvent mixtures. These observations align with earlier studies, where

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hydroethanol and methanol extracts of *P. nigrum* seeds were found to contain phenols and flavonoids, and similar results were noted in *P. betle* leaf extracts (Shetty and Vijayalaxmi, 2012).
The flavonoids and terpenoids were detected in all extracts of both plant species, regardless of solvent polarity, highlighting their broad distribution and ease of extraction across solvent systems. These findings suggest that *P. betle* generally exhibits a higher qualitative presence of phenolic compounds compared to *P. nigrum*, implying that phenolic extraction is more dependent on both the plant matrix and the polarity of the solvent used.

Table 3.1. Qualitative phytochemical analysis of *P. betle* leaves and *P. nigrum* seeds extracts

S. No.	Compounds	B1	B2	B3	P1	P2	P3
1	Phenols	+	+	+	+	-	+
2	Flavonoids	+	+	+	+	+	+
3	Terpenoids	+	+	+	+	+	+

(+) present (-) absent

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)

3.2. Quantitative phytochemical analysis
The quantitative analysis of phytochemicals in *Piper betle* leaves and *Piper nigrum* seeds, extracted using solvents of varying polarity, revealed significant differences in the concentrations of phenols, flavonoids, and terpenes (Figure 3.1; Table 3.2).
Among all tested samples, the hydroethanolic extract of *P. betle* (B1) exhibited the highest phenolic content (787.08 ± 59.32 µg/ml), indicating the superior efficiency of polar: mid polar solvent systems in extracting phenolic compounds. This was followed by the methanolic extract of *P. betle* (B3) and the hydroethanolic extract of *P. nigrum* (P1), with phenolic concentrations of 486.64 ± 20.76 µg/ml and 359.02 ± 5.95 µg/ml, respectively. The methanolic extract of *P. nigrum* (P3) showed moderate phenolic levels (235.97 ± 10.0 µg/ml). In contrast, a substantial decline in phenolic content was observed in the ethanol: hexane extract of *P. betle* (B2) at 111.75 ± 6.51 µg/ml, while *P. nigrum* extracted with the same solvent system (P2) exhibited the lowest phenolic content (20.20 ± 0.57 µg/ml). These findings clearly indicate that *P. betle* generally possesses a higher phenolic content than *P. nigrum*, and that polar solvents such as hydroethanol and methanol are markedly more effective for phenolic extraction.
Similarly, the highest flavonoid concentration was also observed in B1 (*P. betle* – hydroethanol extract) at 576.04 ± 23.56 µg/ml, reinforcing the efficiency of hydroethanol for flavonoid extraction. The methanolic (B3) and ethanol: hexane (B2) extracts of *P. betle* followed with flavonoid contents of 387.15 ± 17.36 µg/ml and 327.60 ± 2.55 µg/ml, respectively. Among the *P. nigrum* extracts, hydroethanol (P1) and methanol (P3) yielded moderate flavonoid levels of 326.71 ± 9.26 µg/ml and 196.60 ± 3.75 µg/ml, respectively. The lowest flavonoid content was recorded in the ethanol:hexane extract of *P. nigrum* (P2) at 131.60 ± 1.13 µg/ml. Overall, *P. betle* extracts, particularly the hydroethanolic (B1), showed significantly higher flavonoid content compared to *P. nigrum*, with polar solvents outperforming non-polar combinations in flavonoid extraction efficiency.
In contrast to phenols and flavonoids, the highest terpene concentration was recorded in the ethanol: hexane extract of *P. nigrum* (P2) at 1027.69 ± 38.77 µg/ml, highlighting the suitability of non-polar solvents for terpene extraction. This was followed by the ethanol: hexane extract of *P. betle* (B2) at 447.69 ± 27.42 µg/ml and the hydroethanolic extract of *P. nigrum* (P1) at 98.90 ± 6.47 µg/ml. Lower terpene contents were observed in the methanolic extract of *P. nigrum* (P3) and the hydroethanolic extract of *P. betle* (B1), with values of 60.72 ± 2.33 µg/ml and 33.75 ± 1.93 µg/ml, respectively. The methanolic extract of *P. betle* (B3) recorded the lowest terpene concentration (18.00 ± 1.18 µg/ml). These results indicate that *P. nigrum*, especially when extracted with non-polar solvents, serves as a richer source of terpenes compared to *P. betle*, and that solvent polarity plays a crucial role in determining terpene extraction efficiency.
The findings of this study are in agreement with previous research, where methanolic extracts of *P. betle* leaves were reported to contain high levels of phenols, flavonoids, and tannins (Basit et al., 2023; Bratati et al., 2022). Likewise, methanolic and hydroalcoholic extracts of *P. nigrum* seeds have demonstrated higher phenolic and flavonoid content compared to aqueous extracts (Ahmad et al., 2015; Zhao et al., 2024), further supporting the influence of solvent polarity on phytochemical yield.

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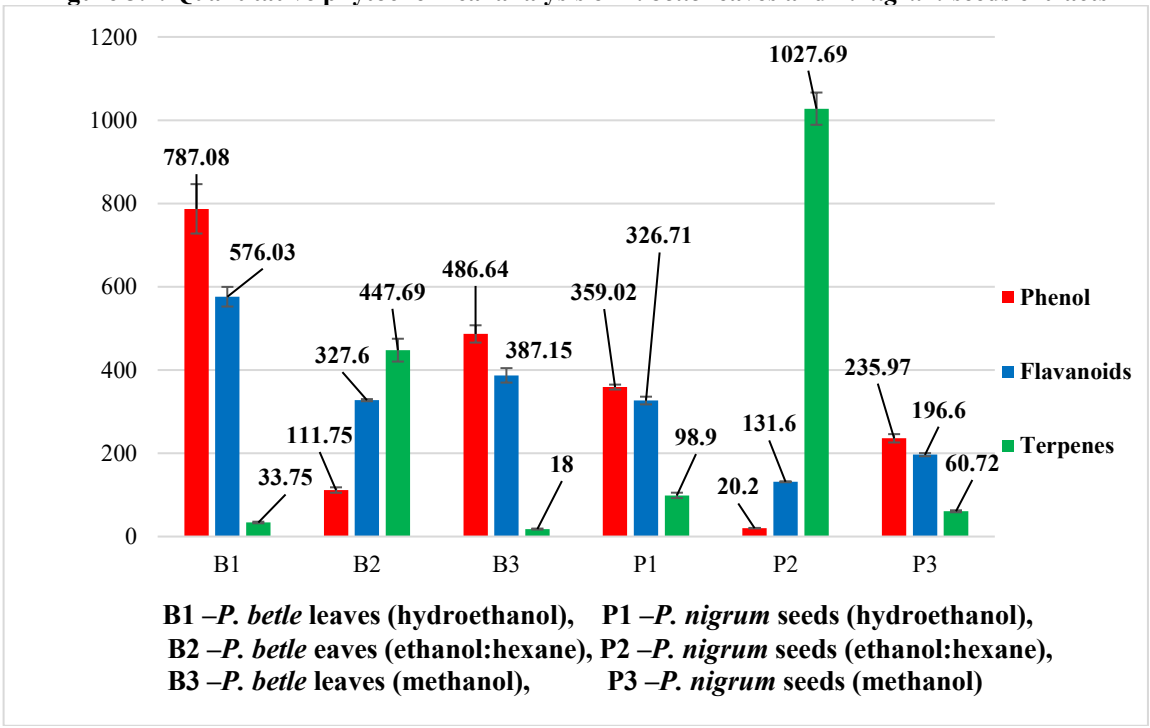
Table 3.2. Quantitative phytochemical analysis of *P. betle* leaves and *P. nigrum* seeds Extracts

Samples	Phenols	Flavonoids	Terpenes
B1 (*P. betle* leaves – hydro ethanol extract)	787.08 ± 59.32	576.04 ± 23.56	33.75 ± 1.93
B2 (*P. betle* leaves –ethanol: hexane extract)	111.75 ± 6.51	327.60 ± 2.55	447.69 ± 27.42
B3 (*P. betle* leaves –methanol extract)	486.64 ± 20.76	387.15 ± 17.36	18.00 ± 1.18
P1 (*P. nigrum* seeds hydro ethanol extract)	359.02 ± 5.95	326.71 ± 9.26	98.90 ± 6.47
P2 (*P. nigrum* seeds –ethanol: hexane extract)	20.20 ± 0.57	131.60 ± 1.13	1027.69 ± 38.77
P3 (*P. nigrum* seeds –methanol extract)	235.97 ± 10.00	196.60 ± 3.75	60.72 ± 2.33

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

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



Based on the results, *Piper betle* extracts, particularly those extracted using hydroethanol and methanol, exhibited a high concentration of phenol and flavonoid compounds, underscoring the effectiveness of polar solvents in extracting antioxidant-rich phytochemicals. In contrast, *Piper nigrum* extracts, especially those extracted with ethanol: hexane, exhibited significantly higher terpene content, indicating the efficiency of non-polar solvents for terpene extraction. These findings highlight the combined impact of plant species and solvent polarity on the phytochemical yield. While *P. betle* exhibited a higher source of bioactive compounds such as phenols and flavonoids, *P. nigrum* proved to be richer in terpenoids, particularly when non-polar solvents are used.

4. Conclusion

This study demonstrates that both plant species and solvent polarity significantly influence the qualitative and quantitative extraction of phytochemicals from *Piper betle* leaves and *Piper nigrum* seeds. Qualitative analysis confirmed the presence of flavonoids and terpenoids across all extracts, while phenolic compounds were more prominent in *P. betle*, particularly when extracted using polar solvents. These observations were further supported by quantitative analysis, where the hydroethanolic extract of *P. betle* (B1) exhibited the highest concentrations of phenols and flavonoids, indicating its potential as a rich source of antioxidant compounds. In contrast, the ethanol: hexane extract of *P. nigrum* (P2) yielded the highest terpene content, underscoring the efficacy of non-polar solvents in terpene extraction.

Overall, the results highlight the pivotal role of solvent polarity in determining phytochemical extraction efficiency. *P. betle* showed the greater suitability for the extraction of antioxidant-rich phenolic and flavonoid compounds, whereas *P. nigrum* is more suitable for terpene recovery. These insights are valuable for guiding solvent selection in phytochemical extraction protocols and can support the development of targeted plant-based therapeutic and nutraceuticals formulations.

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