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Comparative Phytochemical Profiling of *Piper Betle* and *Piper Longum*: A Qualitative and Quantitative Approach

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Abstract

The genus *Piper* includes several medicinally significant species that are extensively utilized in traditional healthcare practices due to their diverse secondary metabolites and associated biological activities. In the present study, a comparative qualitative and quantitative phytochemical evaluation of *Piper betle* and *Piper longum* was carried out to assess solvent- and organ-specific variations in phytoconstituent distribution. Leaves, stems, and roots of both species were subjected to Soxhlet extraction using solvents of different polarities, including hexane, ethyl acetate, chloroform, methanol, and water. Extraction efficiency varied markedly with solvent polarity and plant part, with aqueous extracts yielding the highest extractive values, whereas methanolic extracts exhibited greater phytochemical diversity. Preliminary qualitative screening revealed the presence of major classes of secondary metabolites such as carbohydrates, terpenoids, steroids, flavonoids, tannins, and saponins, predominantly in methanolic and aqueous fractions compared to non-polar solvent extracts. Based on the qualitative profile, methanolic extracts were selected for quantitative estimation of carbohydrates, steroids, and terpenoids using established colorimetric methods, employing glucose, cholesterol, and linalool as reference standards. Quantitative results demonstrated significant interspecific and organ-dependent variations, with stem and root extracts of *Piper longum* showing comparatively higher levels of the quantified phytochemicals than *Piper betle*. Thus, the findings emphasize the phytochemical potential of both *Piper* species and highlight the influence of extraction solvent and plant organ on metabolite composition. This comparative study provides baseline data that may facilitate future bioactivity-guided investigations and detailed compound-level characterization for therapeutic and nutraceutical applications.

Keywords: Phytochemical Analysis, *Piper Betle*, *Piper Longum*, Qualitative, Quantitative.

Introduction

The genus *Piper* (family Piperaceae) represents one of the largest genera of flowering plants, comprising over a thousand species that are primarily distributed in tropical and subtropical regions. Species belonging to this genus have received sustained scientific and ethno botanical attention due to their extensive use as medicinal agents, spices, and economically valuable crops (1). Several *Piper* species form important components of traditional healthcare practices, including Ayurveda, Siddha, Unani, Chinese medicine, and indigenous folk practices, where they are employed for managing a wide range of ailments (2, 3). *Piper Betle* L. is an evergreen perennial climber cultivated extensively for its leaves. The leaves are traditionally consumed in combination with areca nut and slaked lime, and are also utilized in ethno medicine for treating microbial infections, inflammatory conditions, wounds, and digestive disturbances. Earlier phytochemical investigations indicate that *P. betle* leaves possess diverse bioactive constituents, particularly volatile oils and phenolic compounds such as chavicol, chavibetol, eugenol, hydroxychavicol, and piper betol, which are associated with reported anti-inflammatory, antidiabetic, antioxidant, anticancer, and antimicrobial activities. In addition to phenolic constituents, secondary metabolites including flavonoids, tannins, terpenoids, steroids, and alkaloids have also been documented, although their abundance varies with plant organ, extraction solvent, and geographical origin (4, 5). *Piper longum* L. is a medicinally significant species within the genus, traditionally valued for its fruits and roots, with occasional use of the stems. This aromatic perennial climber is widely used in traditional formulations for managing respiratory ailments, gastrointestinal disorders, metabolic diseases, and neurological conditions. The

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pharmacological activities of *P. longum* have been largely attributed to bioactive constituents such as piperine, piperlongumine, volatile oils, lignans, terpenoids, steroids, and other secondary metabolites. Previous studies have demonstrated that different parts of *P. longum* vary considerably in their phytochemical composition, emphasizing its therapeutic potential, including antidiabetic, antiasthmatic, antitumor, and neuroprotective activities (6, 7). Although individual phytochemical and pharmacological studies on *P. betle* and *P. longum* are available, systematic comparative investigations focusing on multiple plant parts using standardized qualitative and quantitative approaches remain limited. Moreover, many existing reports emphasize compound-specific or chromatographic profiling of selected extracts, while comprehensive preliminary phytochemical evaluations across different solvents and plant parts are comparatively scarce. Understanding the qualitative and quantitative differences in their phytochemical profiles is crucial not only for validating their traditional uses but also for guiding the development of standardized herbal formulations and identifying potential leads for pharmaceutical applications (8).

In this context, the present investigation was undertaken to conduct a comparative qualitative and quantitative phytochemical profiling of leaves, stems, and roots of *Piper betle* and *Piper longum*. Soxhlet extraction was performed using solvents of different polarities to assess solvent-dependent variations in phytochemical extraction. The study emphasizes preliminary phytochemical screening and quantitative estimation of selected major classes of secondary metabolites to generate baseline comparative data. The outcomes are intended to elucidate organ- and solvent-specific phytochemical distribution patterns and to provide a scientific foundation for future bioactivity-guided studies and compound-level characterization of *Piper* species.

Materials and Methods

Collection and Authentication of Plant Materials

Healthy and disease-free leaves, stems, and roots of *Piper betle* and *Piper longum* were collected from Mulagumoodu (8°16′3.00″ N, 77°17′28.20″ E), located in Kanyakumari District, Tamil Nadu, India. The collected plant materials were subjected to taxonomic verification and authenticated by Dr. R. Subitha Shajini, Department of Botany, Women's Christian College, Nagercoil, Tamil Nadu, India. Voucher authentication was carried out prior to further experimental processing.

Processing of Plant Materials

After collection, the plant materials were cleaned thoroughly to remove adhering soil particles and extraneous matter by washing under running tap water, followed by rinsing with distilled water. The cleaned samples were shade-dried at room temperature under well-ventilated conditions until desiccated completely. The dried plant parts were then mechanically ground into fine powder using an electric grinder and stored in airtight containers under dry conditions until extraction (9).

Solvent Extraction Procedure

Powdered samples (approximately 20 g each) of leaves (A1), stems (A2), and roots (A3) of *Piper betle*, and leaves (C1), stems (C2), and roots (C3) of *Piper longum* were subjected to Soxhlet extraction. Sequential extraction was performed using solvents of increasing polarity, namely hexane, chloroform, ethyl acetate, methanol, and distilled water. For each extraction, 200 mL of solvent was used, and the extraction temperature was maintained near the respective boiling points of the solvents (hexane and chloroform at 60 °C, ethyl acetate and methanol at 70 °C, and water at 100 °C). The extraction process was continued for a maximum duration of 24 h or until the siphoning solvent appeared colourless. The resulting extracts were concentrated under reduced pressure using a rotary evaporator at temperatures ranging between 40 and 60 °C and subsequently allowed to cool to room temperature. The dried extracts were weighed, and extractive yield was calculated using the following formula [1]:

Percentage Yield (%) = (Weight of dried extract (g) / Weight of plant material (g)) × 100 [1]

Each extract was assigned a specific code: hexane extracts (A1HX, A2HX, A3HX, C1HX, C2HX, C3HX), chloroform extracts (A1CH, A2CH, A3CH, C1CH, C2CH, C3CH), ethyl acetate extracts (A1EA, A2EA, A3EA, C1EA, C2EA, C3EA), methanol extracts (A1MH, A2MH, A3MH, C1MH, C2MH, C3MH), and aqueous extracts (A1AQ, A2AQ, A3AQ, C1AQ, C2AQ, C3AQ). All extracts were stored in airtight containers at 4 °C until further analysis (10). A schematic representation of the extraction and analysis workflow is provided in Figure 1.

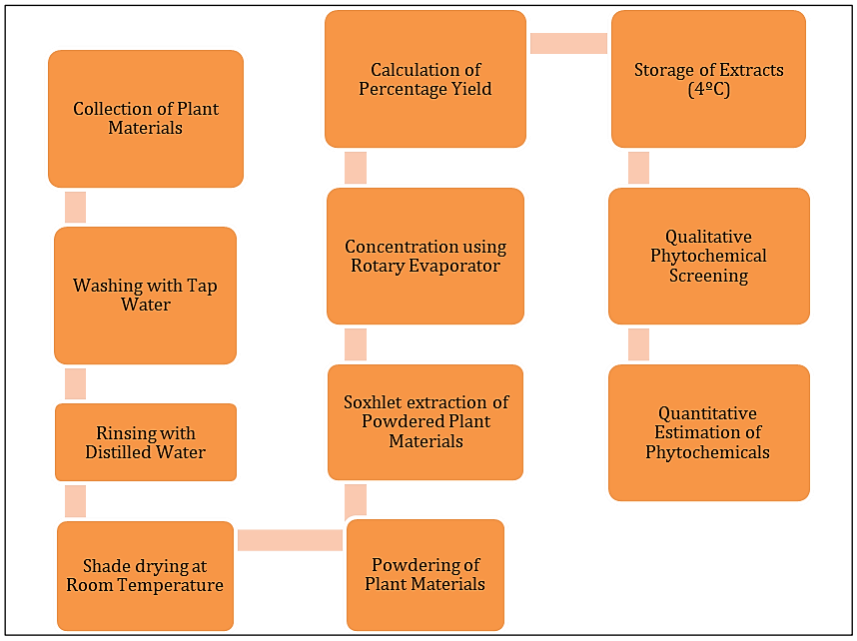

Figure 1: Schematic Representation of Plant Material Processing, Soxhlet Extraction and Phytochemical Analysis of *Piper betle* and *Piper longum*

Qualitative Phytochemical Analysis

Preliminary phytochemical screening of the various solvent extracts was carried out to assess the presence of major classes of primary and secondary metabolites using standard qualitative assays.

Carbohydrates (Benedict's test): An aliquot of 2 mL of each extract was treated with 2 mL of Benedict's reagent and heated in a boiling water bath for approximately 2 min. The development of a brick-red precipitate was considered indicative of reducing carbohydrates (11).

Proteins (Biuret Test): To 3 mL of the extract, a few drops of 4% sodium hydroxide followed by 1% copper sulfate solution were added. Formation of a violet or pink coloration confirmed the presence of proteins (12).

Glycosides (Keller-Kiliani Test): Two millilitres of extract were mixed with glacial acetic acid containing one drop of 5% ferric chloride, followed by careful addition of concentrated sulfuric acid along the test tube wall. The appearance of a reddish-brown ring at the interface with a bluish-green upper layer indicated the presence of cardiac glycosides (13).

Steroids (Salkowski Test): The extract (2 mL) was mixed with chloroform and concentrated sulfuric acid. A red coloration in the chloroform layer accompanied by greenish-yellow fluorescence in the acid layer signified the presence of steroids (13).

Alkaloids (Mayer's Test): One millilitre of extract was acidified with dilute hydrochloric acid and gently heated, followed by the addition of Mayer's reagent. Formation of a yellow precipitate indicated the presence of alkaloids (12).

Flavonoids (Aluminium Chloride test): The extract was treated sequentially with concentrated hydrochloric acid and magnesium turnings, followed by the addition of sodium hydroxide solution. The development of a pink coloration confirmed the presence of flavonoids (14).

Tannins (Lead Acetate Test): A few drops of lead acetate solution were added to 1 mL of extract. Formation of a white precipitate was taken as a positive indication of tannins (15).

Saponins (Foam Test): The extract was vigorously shaken with distilled water. Persistent froth formation was considered evidence of saponins (12).

Phenols (Ferric Chloride Test): Diluted extract was treated with neutral ferric chloride solution. Development of a dark green coloration indicated the presence of phenolic compounds (15).

Terpenoids (Modified Salkowski Test): The extract was mixed with chloroform, followed by careful addition of concentrated sulfuric acid along the sides of the test tube. The appearance of a reddish-brown interface layer confirmed the presence of terpenoids (14).

Quantitative Phytochemical Analysis

Estimation of Carbohydrates: Total carbohydrate content was quantified using the Anthrone method with glucose as the reference standard (16). Extracts were homogenized in 80% ethanol and centrifuged at 4000 rpm for 10 min. An aliquot (0.5 mL) of the supernatant was reacted with Anthrone reagent and heated in a boiling water bath for 15 min. After cooling in the dark, absorbance was measured at 650 nm. A glucose calibration curve (20–200 µg/mL) was prepared ($y = 0.0053x + 0.0008$; $R^2 = 0.998$), and results were expressed as mg glucose equivalents per gram of dried extract (mg GE/g).

Estimation of Steroids: Steroid content was determined using the Zak method, employing cholesterol as the standard (17). The extract (0.5 mL) was reacted with ferric chloride reagent and concentrated sulfuric acid, followed by incubation at room temperature for 30 min. Absorbance was recorded at 540 nm. The cholesterol standard curve (20–200 µg/mL) yielded the regression equation $y = 0.0033x - 0.0214$ ($R^2 = 0.9988$). Results were expressed as mg cholesterol equivalents per gram of dried extract (mg CE/g).

Estimation of Terpenoids: Total terpenoid content was estimated using a modified colorimetric method (18). The extract was mixed with chloroform and allowed to stand, followed by the addition of concentrated sulfuric acid. The reaction mixture was incubated in the dark at room temperature until a reddish-brown precipitate formed. The precipitate was dissolved in methanol, and absorbance was measured at 538 nm. Quantification was performed using a linalool standard curve (20–200 µg/mL) with the regression equation $y = 0.0051x - 0.0305$ ($R^2 = 0.9978$). Results were expressed as mg linalool equivalents per gram of dried extract (mg LE/g).

Results

Yield of Soxhlet Extracted Samples

Soxhlet extraction of different parts of *Piper betle* (A1-A3) and *Piper longum* (C1-C3) using solvents of varying polarity resulted in differential extraction yields (Table 1).

Among the solvents tested, aqueous extracts produced the highest yields in most plant parts. The highest yield was recorded in the aqueous leaf extract of *P. longum* (C1AQ, 32.6%), followed by the aqueous leaf extract of *P. betle* (A1AQ, 32.3%) and the aqueous stem extract of *P. longum* (C2AQ, 30.2%).

Table 1: Yield of Soxhlet-Extracted Samples

Sample Code	Dried Weight of Plant Material (g)	Dried Weight of Plant Extract (g)	% of Yield (%)
A1HX	10	0.45	4.5
A1MH	10	1.86	18.6
A1EA	10	0.92	9.2
A1CH	10	0.78	7.8
A1AQ	10	3.23	32.3
A2HX	10	0.22	2.2
A2MH	5	0.75	15
A2EA	5	0.14	2.8
A2CH	6.6	0.25	3.7
A2AQ	6	1.41	23.5
A3HX	5	0.08	1.6
A3MH	4	0.41	10.25
A3EA	2.4	0.08	3.33
A3CH	2.4	0.09	3.75
A3AQ	2.3	0.48	22.85
C1HX	10	0.5	5.0
C1MH	6	0.87	14.5
C1EA	3	0.181	6.03
C1CH	3	0.23	7.7
C1AQ	3	0.98	32.6
C2HX	10	0.07	0.7
C2MH	10	0.42	4.2
C2EA	10	0.15	1.5
C2CH	10	0.13	1.3
C2AQ	10	3.02	30.2
C3HX	3.03	0.07	2.31
C3MH	3.0	0.33	11
C3EA	3.03	0.12	3.96
C3CH	3.64	0.15	4.12
C3AQ	3.77	0.41	10.87

Methanolic extracts yielded moderate amounts, with notable yields observed in the leaf extract of *P. betle* (A1MH, 18.6%) and the stem extract (A2MH, 15%). Ethyl acetate and chloroform extracts showed relatively lower yields, ranging from 1.3% to 9.2%, while hexane extracts consistently produced the lowest yields. The minimum yield was observed in the hexane stem extract of *P. longum* (C2HX, 0.7%).

Qualitative Phytochemical Screening

Qualitative phytochemical evaluation of the solvent extracts obtained from the leaves, stems, and roots of *Piper betle* and *Piper longum* demonstrated the presence of a wide range of bioactive secondary metabolites. The screening results indicated the occurrence of carbohydrates, glycosides, flavonoids, tannins, alkaloids, saponins, steroids, phenolic compounds, and terpenoids across different solvent extracts. The distribution of these phytoconstituents varied depending on the plant part and the polarity of the extraction solvent. The detailed qualitative profiles of *P. betle* and *P. longum* extracts are presented as shown in Table 2 and Table 3.

Table 2: Qualitative Phytochemical Screening of *Piper betle* Extracts

Extract	Phytochemical tests for									
	CHO	PRO	GLY	STR	AKL	FLV	TNN	SPN	PHE	TER
A1MH	+	-	-	+	-	-	+	-	-	+
A2MH	+	-	-	-	-	+	-	-	-	+
A3MH	+	-	-	-	-	-	+	-	-	+
A1HX	-	-	-	-	-	-	-	-	-	+
A2HX	-	-	-	-	-	-	-	+	-	-
A3HX	-	-	-	+	-	-	-	-	-	+
A1EA	-	-	+	+	-	-	-	-	-	-
A2EA	-	-	+	+	-	-	-	-	-	+
A3EA	-	-	-	+	-	-	-	-	-	+
A1CH	-	-	-	-	+	-	-	-	-	-
A2CH	-	-	-	-	+	-	+	-	-	-
A3CH	-	-	-	+	-	-	-	-	-	+
A1AQ	+	-	-	-	-	-	+	+	-	+
A2AQ	+	-	-	-	+	+	-	+	-	-
A3AQ	+	-	-	-	-	-	+	-	-	-

NB: CHO- Carbohydrates; PRO- Proteins; GLY- Glycosides; STR- Steroids; AKL- Alkaloids; FLV- Flavonoids; TNN- Tannins; SPN- Saponins; PHE- Phenols; TER- Terpenoids; (+)- Present; (-)- Absent

Table 3: Qualitative Phytochemical Screening of *Piper longum* Extracts

Extract	Phytochemical tests for									
	CHO	PRO	GLY	STR	AKL	FLV	TNN	SPN	PHE	TER
C1MH	+	-	-	-	-	-	-	-	-	+
C2MH	+	-	-	+	-	-	+	-	-	+
C3MH	+	-	-	-	-	-	+	-	-	+
C1HX	-	-	+	-	-	-	-	-	-	+
C2HX	-	-	-	+	-	-	-	-	-	+
C3HX	-	-	-	+	-	-	-	-	-	+
C1EA	-	-	-	-	-	-	-	-	-	-
C2EA	-	-	-	+	-	-	-	-	-	+
C3EA	-	-	+	+	-	-	-	-	-	+
C1CH	-	-	+	+	-	-	-	-	-	+
C2CH	-	-	-	+	-	-	-	+	-	+
C3CH	-	-	+	+	-	+	-	-	-	+
C1AQ	+	-	-	-	-	+	+	+	-	-
C2AQ	+	-	-	-	-	-	+	-	-	-
C3AQ	+	-	+	-	-	-	-	+	-	-

NB: CHO- Carbohydrates; PRO- Proteins; GLY- Glycosides; STR- Steroids; AKL- Alkaloids; FLV- Flavonoids; TNN- Tannins; SPN- Saponins; PHE- Phenols; TER- Terpenoids; (+)- Present; (-)- Absent

In *Piper betle*, methanolic extracts (A1MH–A3MH) consistently exhibited the presence of carbohydrates and terpenoids in all examined plant parts. Tannins were detected in the leaf and root extracts, whereas flavonoids were confined to the stem extract. Steroids were observed only in the leaf extract. Aqueous extracts contained carbohydrates along with tannins, saponins, and terpenoids, although their occurrence varied among the different plant parts. In contrast, hexane, ethyl acetate, and chloroform extracts of *P. betle* showed a comparatively restricted phytochemical profile, with terpenoids being the most commonly detected constituents. Notably, saponins were identified exclusively in the aqueous stem extract.

In *Piper longum*, methanolic extracts (C1MH–C3MH) consistently demonstrated the presence of carbohydrates and terpenoids across leaves, stems, and roots, while tannins were predominantly detected in the stem and root extracts. Aqueous extracts exhibited carbohydrates along with flavonoids, tannins, saponins, and glycosides depending on the plant part. Terpenoids were detected across multiple solvent extracts.

Quantitative Phytochemical Screening

In view of the qualitative phytochemical findings, methanolic extracts of leaves, stems, and roots from both *Piper* species were selected for quantitative determination of carbohydrates, steroids, and terpenoids employing established colorimetric methods, as shown in Tables 4-6.

Carbohydrate Content

Carbohydrate content varied among plant parts of both species. The highest carbohydrate concentration was observed in the stem extract of *P. longum* (C2MH, 195.59 mg/g). Leaf extracts of *P. betle* (A1MH, 169.89 mg/g) and *P. longum* (C1MH, 169.33 mg/g) showed comparable values. Lower concentrations were observed in root extracts, with the lowest value recorded in the stem extract of *P. betle* (A2MH, 149.42 mg/g) shown in Table 4.

Table 4: Quantification of Carbohydrates

Plant Extract	Absorbance at 650 nm	Carbohydrate content (mg/g)
A1MH	0.908	169.89
A2MH	0.796	149.42
A3MH	0.871	163.13
C1MH	0.905	169.33
C2MH	1.049	195.59
C3MH	0.609	115.34

Table 5: Quantification of Steroids

Plant Extract	Absorbance at 538 nm	Steroid content (mg/g)
A1MH	0.135	48.21
A2MH	0.169	58.26
A3MH	0.199	67.30
C1MH	0.187	63.73
C2MH	0.237	78.31
C3MH	0.163	56.37

Steroid Content

Steroid concentration varied among the methanolic extracts. The stem extract of *P. longum* (C2MH) exhibited the highest steroid content (78.31 mg/g). In *P. betle*, the highest steroid content was observed in the root extract (A3MH, 67.30 mg/g), followed by the stem and leaf extracts shown in Table 5.

Terpenoid content

Terpenoid estimation revealed that the root extract of *P. longum* (C3MH) contained the highest terpenoid concentration (33.21 mg/g). In *P. betle*, the stem extract (A2MH) showed the highest terpenoid content (21.50 mg/g) while the leaf and root extracts exhibited comparable levels shown in Table 6.

Table 6: Quantification of Terpenoids

Plant Extract	Absorbance at 538 nm	Terpenoid content (mg/g)
A1MH	0.055	18.50
A2MH	0.073	21.50
A3MH	0.044	17.65
C1MH	0.072	21.33
C2MH	0.057	18.74
C3MH	0.135	33.21

Discussion

The extraction yield of *Piper betle* and *Piper longum* varied markedly with solvent polarity and plant part, underlining the influence of extraction conditions on phytochemical recovery. The consistently higher yields obtained with aqueous and methanolic solvents indicate a predominance of polar and moderately polar constituents in both species. Polar solvents such as water are known to preferentially solubilize highly polar constituents, including carbohydrates, glycosides, tannins, and saponins. In contrast, methanol, because of its

moderate polarity, enables the extraction of a wider spectrum of secondary metabolites, notably terpenoids and steroids (19, 20). This solvent-dependent extraction behavior is consistent with the findings of the present investigation, wherein aqueous extracts produced the highest yields, followed by methanolic extracts.

In contrast, the relatively low yields observed in hexane, ethyl acetate, and chloroform extracts suggest a lower abundance of non-polar phytoconstituents, particularly in stem and root tissues. The generally higher extractive yields obtained from leaves compared to stems and roots may reflect their physiological role as primary sites of photosynthesis and secondary metabolite biosynthesis, a trend commonly reported for medicinal plants (21-23).

Qualitative phytochemical screening further demonstrated that methanolic and aqueous extracts possess a richer and more diverse phytochemical composition than non-polar solvent extracts. The detection of carbohydrates, flavonoids, tannins, saponins, steroids, and terpenoids predominantly in polar extracts aligns with previous reports on *P. betle*, where polar solvents were shown to extract a wide range of bioactive constituents from leaves and stems (24, 25). The selective detection of terpenoids in hexane and ethyl acetate extracts supports solvent-specific solubility and highlights the chemical diversity of these metabolites (26).

In *P. longum*, the widespread detection of terpenoids across multiple solvents and plant parts suggests their extensive distribution within the species. The absence of alkaloids in methanolic extracts, despite earlier reports indicating their presence, may be attributed to geographical origin, seasonal variation, plant age, or chemotypic differences, factors known to influence phytochemical composition in medicinal plants. Such variability emphasizes the importance of region-specific phytochemical evaluation (27-29).

Quantitative analysis confirmed that carbohydrates, steroids, and terpenoids are abundantly present in the methanolic extracts of both *P. betle* and *P. longum*. Notably, *P. longum* exhibited higher carbohydrate and steroid contents, particularly in stem and root extracts, indicating interspecific and organ-specific differences in metabolite accumulation (30, 31). The elevated terpenoid content observed in root extracts may be associated with their roles in defense, storage, and interaction with the rhizosphere (32).

The predominance of bioactive secondary metabolites in methanolic and aqueous extracts thus supports the ethno medicinal relevance of *Piper* species and reinforces their phytochemical potential for therapeutic and nutraceutical applications. The comparative qualitative and quantitative data generated in this study provide baseline phytochemical information and serve as a foundation for future bioactivity-guided studies and compound-level characterization of *P. betle* and *P. longum*.

Conclusion

This study offers a detailed comparison of the phytochemical profiles of *Piper betle* and *Piper longum*, emphasizing extractive yields and the quantitative assessment of major bioactive compounds, including terpenoids, steroids, and carbohydrates. Among the solvents used, methanol consistently demonstrated superior extraction efficiency across all plant parts, with aqueous extracts also showing high yields in specific cases. Quantitative phytochemical analysis revealed that *Piper longum* methanolic extracts generally contained higher levels of steroids, terpenoids, and carbohydrates compared to *Piper betle*, particularly in stem and root tissues. The high content of these phytochemicals, known for their therapeutic relevance, supports the ethnomedicinal use of these species and highlights their potential in pharmacological and nutraceutical applications. These findings lay a strong foundation for future research, including bioactivity-guided fractionation and molecular characterization of active constituents. Further investigations focusing on antioxidant, antimicrobial, and cytotoxic activities are warranted to substantiate the therapeutic relevance of the identified phytochemicals and to facilitate the development of scientifically validated plant-based formulations.

Abbreviations

None.

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Author Contributions

Jasmine F: conceptualization of the study, execution of experiments, data acquisition, analysis, interpretation of results, preparation of the original manuscript, approved the final version of the manuscript, K R Beula Rani: contributed to the study design, supervised data analysis, interpretation, critically reviewed, edited the manuscript, approved the final version of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest associated with this study.

Declaration of Artificial Intelligence (AI) Assistance

Generative artificial intelligence tools (OpenAI) were utilized solely for language editing and improving the clarity and readability of the manuscript. No AI tools were used in study design, experimental procedures, data generation, analysis, or interpretation. The authors take full responsibility for the accuracy, originality, and integrity of the content presented.

Ethics Approval

Ethical approval was not required for this study, as it did not involve human participants or animal experimentation.

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References

1. Arvinder K, Sohpal VK, Zabeer A. *Piper mullesua*: Traditional uses and pharmacological potential. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):450-3.
2. Salehi B, Zakaria ZA, Gyawali R, Ibrahim SA, Rajkovic J, Shinwari ZK, Khan T, Sharifi-Rad J, Ozleyen A, Turkdonmez E, Valussi M, Tumer TB, Monzote Fidalgo L, Martorell M, Setzer WN. *Piper* species: A comprehensive review on their phytochemistry, biological activities and applications. Molecules. 2019; 24(7):1364.
3. Habib A, Arzoo KS, Kalam MA, Aziz A, Babu C, Ahmad B. Exploring the medicinal benefits of filfil Daraz (*Piper longum* L.): Insights from Unani medicine and modern perspectives. Journal of Complementary and Alternative Medical Research. 2024;25(12):133-145.
4. Biswas P, Anand U, Saha SC, Kant N, Mishra T, Masih H, Bar A, Pandey DK, Jha NK, Majumder M, Das N, Gadekar VS, Shekhawat MS, Kumar M, Radha, Prockow J, Lastra JMP, Dey A. Betel vine (*Piper betle* L.): A comprehensive insight into its ethnopharmacology, phytochemistry, and pharmacological, biomedical and therapeutic attributes. Journal of Cellular and Molecular Medicine. 2022;26(11):3083-119.
5. Bhalerao SA, Verma DR, Gavankar RV, Teli NC, Rane YY, Didwana VS, Trikannad A. Phytochemistry, pharmacological profile and therapeutic uses of *Piper betle* Linn. – An overview. Research and Reviews: Journal of Pharmacognosy and Phytochemistry. 2013;1(2):10-9.
6. Grover M. *Piper longum* (Pippalimool): A systematic review on the traditional and pharmacological properties of the plant. World Journal of Pharmaceutical and Medical Research. 2021;7(8):281-9.
7. Biswas P, Ghorai M, Mishra T, Gopalakrishnan AV, Roy D, Mane AB, Mundhra A, Das N, Mohture VM, Patil MT, Rahman H, Jha NK, Batiha GES, Saha SC, Shekhawat MS, Radha, Kumar M, Pandey DK, Dey A. *Piper longum* L.: A comprehensive review on traditional uses, phytochemistry, pharmacology, and health promoting activities. Phytotherapy Research. 2022;36(12):4425-76.
8. Gautam PK, Vijaya S, Patil DN, Barwant MM, Narkhede S. Phytochemical analysis of medicinal plants used in traditional healing practices. Revista Electronica de Veterinaria. 2024;25(2):235-9.
9. Ghosh D, Mondal S, Ramakrishna K. Pharmacobotanical, physicochemical and phytochemical characterisation of a rare salt-secreting mangrove *Aegialitis rotundifolia* Roxb. (*Plumbaginaceae*) leaves: A comprehensive pharmacognostical study. South African Journal of Botany. 2017; 113:212-29.
10. Chaudhary NK, Chaudhary A, Shakya M, Limbu DK, Oli PS. Phytochemical screening and antimicrobial activity of *Piper betle* L. and *Nicotiana tabacum* L. across Dharan, Nepal. Himalayan Journal of Science and Technology. 2023;7(1):87-100.
11. Brain KR, Turner TD. Practical evaluation of phytopharmaceuticals. 1st ed. Bristol: Wright-Scientechnica; 1975. p. 144. ISBN: 9780723603126.
12. Ansari SH. Essentials of pharmacognosy. 1st ed. New Delhi: Birla Publications; 2006. p. 357-590. ISBN: 9788186270288.
13. Shrestha P, Adhikari S, Lamichhane B, Shrestha BG. Phytochemical screening of the medicinal plants of Nepal. IOSR Journal of Environmental Science, Toxicology and Food Technology. 2015;1(6):11-7.
14. Harborne JB. Phytochemical methods. 2nd ed. London: Chapman and Hall Publications; 1984. p. 288. ISBN: 9780412574300.
15. Mukherjee PK. Quality control of herbal drugs. 1st ed. New Delhi: Business Horizons Pharmaceutical Publishers; 2002. p.356-358. ISBN: 9788178330128.
16. Roe JH. The determination of sugar in blood and spinal fluid with Anthrone reagent. Journal of Biological Chemistry. 1955;212(1):335-43.

17. Zak B, Dickenman RC, White EG, Burnett H, Cherney PJ. Rapid estimation of free and total cholesterol. American Journal of Clinical Pathology. 1954;24(11):1307-15.
18. Ghorai N, Chakraborty S, Gucchait S, Saha SK, Biswas S. Estimation of total terpenoids concentration in plant tissues using a monoterpene, linalool as standard reagent. Nature Protocol Exchange. 2012;1-6. https://www.nature.com/protocolexchange/protocols/2529
19. Khatnal P, Verma RK, Sharma MK. Qualitative analysis of crude extract from the leaves of *Cassia occidentalis*. Journal of Science Innovations and Nature of Earth. 2024;4(4):75-80.
20. Turay A, Kargbo HA. Solvent efficiency in phytochemical extraction: A comparative study of methanol and water in *Cassia siamea*. International Journal of Innovative Science and Research Technology. 2025;10(5):462-8.
21. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017; 6(4):01-23.
22. Yuan F, Yan S, Zhao J. Elucidating the phytochemical landscape of leaves, stems, and tubers of *Codonopsis convolvulacea* through integrated metabolomics. Molecules. 2024; 29(13):01-19.
23. Asmah N, Suniarti DF, Margono A, Mas'ud ZA, Bachtiar EW. Identification of active compounds in ethyl acetate, chloroform, and N-hexane extracts from peels of *Citrus aurantifolia* from Maribaya, West Java, Indonesia. Journal of Advanced Pharmaceutical Technology and Research. 2020;11(3):107-12.
24. Biswal S. Phytochemical analysis and a study on the antiestrogenic antifertility effect of leaves of *Piper betle* in female albino rat. Ancient Science of Life. 2014;34(1):16-22.
25. Syahidah A, Saad CR, Hassan MD, Rukayadi Y, Norazian MH, Kamarudin MS. Phytochemical analysis, identification and quantification of antibacterial active compounds in betel leaves, *Piper betle* methanolic extract. Pakistan Journal of Biological Sciences. 2017;20(2):70-81.
26. Siddiqui T, Khan MU, Sharma V, Gupta K. Terpenoids in essential oils: Chemistry, classification, and potential impact on human health and industry. Phytomedicine Plus. 2024; 4(2):01-25.
27. Irawan C, Tambunan JA, Rachmy S, Putri ID, Rosalina, Suhartini. Phytochemical analysis of red betel (*Piper crocatum* Ruiz and Pav) stem extracts and its antioxidant and alpha-glucosidase inhibitory potentials. Tropical Journal of Natural Product Research. 2024;8(4):7042-8.
28. Rajan P, Devi CA, Iyer P. Phytochemical analysis of medicinal plants used in the treatment of tuberculosis. International Advanced Research Journal in Science, Engineering and Technology. 2021;8(8):30-5.
29. Sultana NA, Zilani NH, Taraq KT, Al-Din K. Phytochemical, antibacterial and antioxidant activity of *Piper longum* leaves. Pharmacology Online. 2019; 1:27-35.
30. Parrish AN, Samec ILD, Lange BM. Differential accumulation of metabolites and transcripts related to flavonoid, styrylpyrone, and galactolipid biosynthesis in Equisetum species and tissue types. Metabolites. 2022; 12(5):01-18.
31. Weinhold A, Doll S, Liu M, Schedl A, Poschl Y, Xu X, Neumann S, van Dam NM. Tree species richness differentially affects the chemical composition of leaves, roots and root exudates in four subtropical tree species. Journal of Ecology. 2022;110(1):97-116.
32. Zambelli A, Nocito FF, Araniti F. Unveiling the multifaceted roles of root exudates: Chemical interactions, allelopathy, and agricultural applications. Agronomy. 2025;15(4):01-30.

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