





Phenolic compounds from *piper* plants: A review on AI integration for extraction, quantification, antioxidant, antimicrobial properties, and food applications

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ABSTRACT

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Medicinal plants are rich in phenolic compounds, which are commonly found in food and nutraceutical products. The *Piper* plants contain a vast number of bioactive compounds, with phenolics being the most abundant and biologically necessary plant metabolites. The review assessed recent discussions on the various extraction techniques applied to extract phenolic compounds from *Piper* species. To enhance the extraction efficiency of *Piper* plants, innovative extraction methods are employed. Extraction methods such as microwave-assisted, ultrasonic-assisted, and enzyme-assisted extraction improve *Piper* extraction by utilizing low temperatures and reducing extraction durations. AI-driven optimization techniques are suggested to predict the optimal conditions. Reviewing the phenolic compounds extracted from *Piper* plants enhances a better understanding of the extraction process. Phenolic compounds found in *Piper* are renowned for their antioxidant and antimicrobial properties due to the presence of phenolic hydroxyl groups in ring structures. The antioxidant and antimicrobial properties of *Piper* also address the challenges in utilizing its phenolic compounds for biological purposes. The advanced AI-based analytical models are potential in the quantification of phenolic compounds by chromatographic and spectrophotometric methods. The *Piper* plants may become a promising source of phenolic compounds for use in the food industry upon diversification of extraction methods and quantification techniques.

Contribution/Originality: This review assesses current discussions on *Piper* plants. The recent advancements in extraction, identification, and quantification methods to valorize the *Piper* plants, including artificial intelligence, are discussed. This review highlights the potential of antioxidant and antimicrobial properties in *Piper* plants. The challenges in utilizing its phenolic compounds for biological purposes, which extend to their potential for food applications, are reviewed.

1. INTRODUCTION TO PHENOLIC COMPOUNDS IN PIPER PLANTS

Nowadays, food technologists have shown their scientific concern for *Piper* plants due to a variety of phytopharmaceutical advantages, including antimalarial (Yimtchui et al., 2024), antibacterial (Krishana et al., 2024;

Pammi et al., 2024), antioxidant (Krishana et al., 2024; Pammi et al., 2024), anti-Alzheimer (Muthusamy et al., 2024), anti-inflammatory (Yimtchui et al., 2024), antidiabetic (Mingmuang et al., 2024), and anticancer (Krishana et al., 2024). Several studies have shown that *Piper* species are aromatic plants that are extensively cultivated for their seeds and leaves, which are easily identifiable by their odor, making them important spices (Yimtchui et al., 2024). Moreover, it is popular to grow *Piper* species for their seeds and leaves, which have a distinctive aroma, making them valuable spices for culinary and traditional medicine.

Some of well-known species of *Piper* include *Piper colubrinum*, *Piper retrofractum* Vahl, *Piper sarmentosum* Roxb, and *Piper wallichii* (Miq) Hand. Moreover, other notable species are *Piper longum* L., *Piper gaudichaidianum*, *Piper betle* L., *Piper nigrum* L., and *Piper chaba* W. Hunter (Cucinotta et al., 2024; Hemel et al., 2024; Mingmuang et al., 2024; Sruthi & Zachariah, 2024). *Piper* species have also been used extensively in traditional medicine for treating rheumatism, scabies, liver, diarrhea, wound healing, and various other ailments (Yimtchui et al., 2024). While *Piper* plant species have a wide range of medical use and several separated chemicals, little research has been conducted on their extraction and chemical contents, particularly to find bioactive compounds that responsible for therapeutic properties. From a study conducted by Hemel et al. (2024). *P. chaba* were regarded as an important agroforestry species. This might be due to this plant easily to cultivate and suitable for economic sustainability. However, the chemical and functional qualities of *Piper* plants are greatly impacted by abiotic elements such as humidity, climate, season, precipitation, and the dry and wet seasons (Cucinotta et al., 2024).

Piper plants contain a range of primary metabolites including total protein, total carbohydrates, reducing sugars, starch, total fat, and total free amino acids (Sruthi & Zachariah, 2024). Moreover, functional extracts from *Piper* primarily contain secondary metabolites, including terpenes, alkaloids, lipids, and phenolic compounds. An aromatic plant, such as *Piper*, is a popular source of phenolic compounds, which are biologically necessary plant metabolites. Phenolic compounds are commonly used in several industries, including food, traditional medicine, and pharmaceuticals. There is growing evidence that phenolic compounds, particularly in functional foods, may regulate physiological activity and contribute to human health. Furthermore, it is noteworthy that phenolic compounds from *Piper*, particularly those from *Piper* leaves, have been reported to have anti-amylase and anti-lipase characteristics (Mingmuang et al., 2024). There is a belief that phenolic compounds serve as a survival mechanism for plants when they are exposed to varying environmental conditions. Depending on the exposure, phenolic compounds can be classified as flavonoids, phenolic acids, stilbenes, lignans, tannins, curcuminoids, isoflavonoids, lignins, coumarins, and phenolic glycosides. A phenol is identified by a dominating hydroxyl group over an aromatic ring in its structure. In aliphatic alcohols, hydroxyl groups attach to carbon chains. As a result of the instability of their hydroxyl groups, which are associated with aromatic rings, phenols are classified as weak acids. Moreover, phenolic acids can be present in both free and bound forms (Saini et al., 2024). In addition to myrtenol, eugenol, safrole, riboflavin, eucalyptol, allylpyrocatechol, bicyclogermacrene, 3-carene, spathulenol, and retinol, the *Piper* plant also contains compounds like eugenol, myrtenol, riboflavin, and safrole, contributing to its wide application and beneficial properties, including immune function regulation. The study on phenolic compounds is conducted extensively using various extraction techniques. It examines the extraction technique, identification, quantification, and antioxidant and antimicrobial properties of phenolic compounds from *Piper* plants, in addition to their antibacterial and antioxidant qualities in vitro.

2. ARTIFICIAL INTELLIGENCE (AI) FOR EXTRACTING *PIPER* SPECIES

Artificial intelligence (AI) has become a revolutionary technology with important benefits in the food industry, especially in food-based plant extraction, quantification of plant bioactive compounds, food antioxidants, biological properties, and their application in food products. Conventional extraction methods such as plant solvent extraction and steam distillation have limitations due to their inefficiency, degradation of bioactive compounds, and time consumption. Artificial intelligence has introduced new ideas to improve extraction techniques for obtaining bioactive

compounds, particularly from *Piper* species (Pravin & Deepa, 2023). AI optimization techniques, especially deep learning and machine learning, enhance predictive modeling for optimization of *Piper* plant extraction. For example, AI algorithms have the ability to analyze large datasets on various extraction parameters. The extraction parameters such as temperature, pressure, solvent type, and exposure time evaluate the most optimal method for maximizing recovery yield and efficiency of bioactive compounds. In addition to classic methods, AI techniques such as artificial neural networks and genetic algorithms assist in the enhancement of traditional extraction techniques, which contribute to increased extraction performance and reduction in resource consumption (Pravin & Deepa, 2022).

Moreover, the development of AI-integrated extraction systems enhances the automated process control in extraction involving real-time data monitoring and automatic optimizations. For instance, the integration of AI with the Internet of Things facilitates monitoring various extraction parameters such as solvent concentration, temperature, and pH levels. This level of automation allows for tighter control over the extraction processes of important bioactive compounds, including piperine and flavonoids, which significantly improves their efficiency and compound stability (Pravin & Deepa, 2022). AI-assisted bioactive and phytochemical analysis has also proven important in aiding the identification of plants. More sophisticated models based on deep learning can enhance the identification and quantification processes of bioactive constituents of *Piper* species. AI, in conjunction with advanced spectroscopic and chromatographic techniques such as HPLC and FTIR imaging and spectral analysis, can also provide more precise and faster classification of the extracted compounds, which diminishes the errors associated with human operators and the time spent on analysis (Pravin & Deepa, 2023).

Recently, artificial intelligence is responsible for enhancing the environmentally friendly and sustainable extraction techniques of *Piper* species. Through the optimization of green solvents, AI reduces the use of harmful chemicals in advanced extraction methods such as supercritical CO₂ extraction and ultrasound-assisted extraction. Machine learning algorithms show the potential to optimize different extraction conditions to determine the most efficient, sustainable, and cost-effective extraction strategy (Pravin & Deepa, 2022). Moreover, future perspectives of artificial intelligence for extracting *Piper* species have become a main consideration. The application of AI in *Piper* plant extraction presents numerous opportunities, yet challenges remain. The need for extensive datasets, high computational power, and experimental validation of AI-predicted results poses significant hurdles. Future research should aim to refine AI models to further enhance efficiency, sustainability, and commercialization in the pharmaceutical, food, and cosmetic industries. The Table 1 shows the potential AI-based approaches for extracting *Piper* species.

3. METHOD FOR EXTRACTING PHENOLIC COMPOUNDS FROM *PIPER* PLANTS

In general, there are two categories of extraction techniques used for extracting phenolic compounds: traditional and non-conventional. To extract the targeted compounds, the traditional extraction technique uses the concept of solubility of solvents. By using advanced technology, the non-conventional approach is the favored alternative for extracting phenolic compounds. To obtain phenolic compounds, extraction, purification, concentration, and drying are required. A study conducted on the quality of *Piper* plants really depends on processing treatment. Therefore, artificial intelligence techniques offer a solution in plant extraction by optimizing *Piper* plants for enhancing extraction efficiency, extract yield, and quality of bioactive compounds. To maximize the yield and quality of phenolic extraction, it is important to use suitable methods. Based on this extraction method, Figure 1 shows the phenolic compounds extracted from *Piper* plants.

Table 1. Potential AI-based approaches for extracting *Piper* species.

| Processes | Benefit of AI extraction of <i>Piper</i> plant |
|---------------------------------|--|
| Optimizing extraction processes | i) Ability to handle large dataset ii) Combining several extraction parameters iii) AI algorithms can analyze the best plant species for optimal bioactive compounds. iv) AI prediction model will recommend the suitable extraction method vi) AI can detect the impurities in raw plant material and extract |
| Plant compound identification | i) Process image of <i>Piper</i> species rapidly ii) Identify pattern and unique plant characteristics iii) AI approaches can be implemented in various settings, utilizing information from worldwide herbarium collection iv) Consistency in classification and nomenclature v) Minimize weaknesses in manual identification, including specimen collection, labor intensity, time consumption, and environmental disruption. vi) Ability to predict bioactive compounds present in <i>Piper</i> plant. vii) Solve the human deficiency in plant classification and identification |
| Predicting plant yield | i) Accurate in predicting plant yield ii) Reduce computational cost by lowering the dimensionality of the pre-processed image while preserving essential information iii) AI can compare the historical data and current data to improve the extraction yield |
| Automation and robotic | i) Improve <i>Piper</i> extraction to improve production and quality of extract. ii) Effective information to extraction management. |
| Sustainability | i) AI has potential to suggest green solvent ii) AI algorithms may reduce energy consumption iii) Prediction on potential of byproduct utilization iv) AI may suggest the methods to convert <i>Piper</i> waste into bioactive compounds via alternative method such as fermentation |

Source: Pravin and Deepa (2022).

3.1. The Traditional Extraction Method

Traditional extraction methods have been extensively investigated for extracting bioactive compounds such as phenolics in recent decades. Many traditional extraction techniques have been reported and recommended for extracting *Piper* plants. The extraction method plays a crucial role in obtaining phenolic compound-rich extracts. As it offers simple techniques, traditional extraction methods such as organic solvent extraction and hydrodistillation are still widely used in various industries. Researching scientific databases will reveal a number of articles related to maceration. Solid-liquid extraction occurs when a *Piper* plant is immersed in a solvent. Soaking *Piper* plants in organic solvents enhances the extraction process of the phenolic compounds. The extraction yield is highly influenced by various operational parameters, including solvent, extraction temperature, pressure, concentration, pH, moisture, particle size, and raw material type. In obtaining significant phenol yields, solvent selection is the most important criterion in phenolic compound recovery. Water, ethanol, and methanol are the most commonly used solvents. Researchers prefer ethanol over methanol, possibly due to environmental concerns and toxicity issues. Scientific research also uses solvents such as petroleum ether, n-hexane, acetone, ethyl acetate, and chloroform. Researchers [Al-Mamun, Maniruzzaman, Badal, and Haque \(2024\)](#) demonstrated a new antioxidant and antimicrobial technique using n-hexane, ethyl acetate, and methanol as solvents.

The maceration technique is conducted without any high-end instrumentation. Because of this, maceration has become a popular method for extracting phenolic compounds, particularly from *Piper* plants. To enhance the extraction, it is important to stir the plant to ensure the solvent and *Piper* plant are well mixed during maceration, which can take a long time. On the other hand, the Soxhlet extraction method has become a reference method. However, important limitations dominate maceration methods, including exposing the *Piper* sample to high temperatures, which leads to the deterioration of functional bioactive compounds. At higher temperatures, greater solvent solubility can be achieved. Heat is applied to plant material in a process known as decarboxylation ([Rantaša, Slaček, Knez, & Marevci, 2024](#)). Decarboxylation may contribute to various chemical structure changes. These problems will be solved by utilizing advanced extraction methods to rupture the cell walls, enhancing the mass solute transfer from plant materials to solvents. In addition to improving the extraction yield, cutting-edge extraction techniques can improve physicochemical properties, phytopharmaceutical properties, and extraction efficiency.

3.2. Non-Conventional Extraction

With the advent of modern extraction techniques, phenolic compounds can now be extracted from plant material in a wide variety of ways. Modern extraction methods have made a remarkable contribution to the food, cosmeceutical, nutraceutical, and pharmaceutical industries. The equipment now offers researchers multiple options for improving the quantity and quality of bioactive compounds. Modern extraction methods include microwave-assisted extraction, ultrasonic-assisted extraction, and enzymatic extraction. There has been a noticeable reduction in the time for the extraction process and an increase in the yield of bioactive compounds, especially phenolic compounds. Among industry stakeholders, non-conventional extraction is becoming the main focus due to its ability to accelerate the extraction process. The *Piper* extraction has been improved by combining different extraction methods. Single extraction methods were used before the development of combinations of different modern extraction techniques. However, the potential of combinations of different extraction methods has triggered researchers' interest to explore deeper, especially regarding the efficacy of extraction.

Compared to traditional extraction methods, non-conventional methods produce a higher yield of phenolic compounds in a shorter time while using fewer solvents and less energy, which can be described as a simple, rapid, environmentally friendly, and efficient extraction process. Through rapid changes in pressure, enzymes, heat, ultrasonic, and microwave technology, these emerging advanced green extraction technologies modify the structure of plant phenolic compounds, changing their properties to produce highly stable compounds of high quality.

Ultrasound-assisted extraction reveals an interesting phenomenon: plant cell walls can be breached using this extraction method.

3.3. Ultrasonic-Assisted Extraction

Because of its reduced energy consumption, shorter processing time, lower solvent usage, higher extraction rate, and cheaper cost, ultrasonic-assisted extraction has become a viable alternative to traditional extraction techniques. Ultrasonic-assisted extraction makes use of acoustic cavitation as its main driving force. This approach enhances the mechanical interaction between the commercially important enzymes and their substrates using cavitation techniques. Ultrasound increases the ease of release of phenolic compounds from cell materials. During the disruption of plant matrix cells, phenolic compounds are released by cavitation. Besides improving hydration, surface area enlargement as a result of reduced particle size also occurs due to cavitation. In the cytoplasm of the cells, smaller particles that inhibit the formation of phenolic compounds by extracting liquids pose less resistance. The direct contact between the solvent and intracellular cellular constituents is improved, which enhances solvent penetration and mass transfer, thus aiding cavitation. Cell membranes are fragmented by ultrasonic waves as a result of sonic waves generating pressure changes that create cavitation bubbles. Ultrasonic-assisted extraction is also affected by other factors such as ultrasonic power, frequency, solvent, and the ratio of solvent to the *Piper* plant components. Singh et al. (2024) conducted an investigation into ultrasound-assisted phytochemical extraction from *P. betel*. Using the Box-Behnken design, they optimized the solid-solvent ratio, temperature, and time. According to Singh et al. (2024), ultrasound-assisted extraction was used to extract phytochemicals from *P. betle*. The plant was collected from Paan Mandi in Laxmi Bazaar, Naka Hindola, and Lucknow, India. To optimize the process, the researchers used a Box-Behnken experimental design, which helped determine the ideal exposure time, extraction time, and solid-solvent ratio. The study found that ultrasound extraction is more popular than Soxhlet extraction in the food industry because it is efficient, quick, uses less solvent, and consumes less energy. For the extraction, they used 10 grams of betel leaf powder with a maximum extraction time of 30 minutes. The highest total phenolic compounds were obtained for 192 mg GAE/g. This high extraction output might be due to the ultrasound creating cavity bubbles that facilitate solvent transfer from *Piper* samples to the selected solution. The cell wall pore of a *Piper* plant is enlarged, swelled, and hydrated, which enhances the diffusion and movement of mass transfer. The yield was considered reasonable and exhibited a range of 6.5% to 18.86%. This yield is consistent with research conducted by Tran, Tran, and Le (2025), which proves a yield of 17.466%. However, this study exhibited higher total phenolic content (261.904 mg GA/g) compared to the study by Singh et al. (2024) (192 mg GAE/g). This might be due to different geographical collection areas.

3.4. Microwave-Assisted Extraction

Traditional heating methods consume more energy and are less controllable than microwaves. Microwave-assisted extraction is one of the preferred solutions. By penetrating into the materials, the electric field achieves rapid heating and uniform temperature distribution. In addition to enhancing energy efficiency, microwave-assisted extraction enhances solvent purity by reducing solvent consumption. The extraction technique enhances phenolic extraction yields, decreases solvent usage, and shortens reaction times by focusing on the breakdown of plant cell wall integrity, which increases the solubility and diffusion between solvents and intracellular chemicals. Solvent molecules vibrate when microwave irradiation is applied, which leads to electromagnetic field frequencies. Heat and friction are produced during the extraction process, which helps to speed up the transfer of mass and improve the extraction of solutes. As a result, the plant cells break open, allowing the compounds to leak into the surrounding solution. In a study conducted by Olalere, Abdurahman, bin Mohd Yunus, Alara, and Akbari (2018), microwave reflux extraction was used to extract piperine-oleoresin from the *Piper nigrum* (black pepper). Besides that, to optimize the

microwave extraction process, the researchers tested several factors, including microwave power, irradiation time, feed-solvent ratio, and particle size.

Silver nanoparticles from *P. longum* fruit extract can be employed using microwave techniques as a sensitive fluorescent nanoprobe for anticonvulsants such as carbamazepine and risperidone, as studied by Magdy, Aboelkassim, El-Domany, and Belal (2024). The microwave technique is currently the recommended method for extracting *Piper* bioactive components, mainly because of its high extraction efficiency. The microwave extraction method is inexpensive and simple to use. The phytochemical content of *P. nigrum* demonstrated strong inhibition against α -glucosidases to strengthen the immune system, according to a study by Pothipongsa, Phuwapraisirisan, and Damsud (2024) and Pothipongsa et al. (2024). Additionally, the study suggested that the best solvent for microwave extraction is water. This may be because *Piper* plant extract can be added straight to food and herbal products without raising any concerns about solvent residue toxicity. The study shows the optimum condition for microwave energy at 500 W for 30 seconds, yielding the highest total phenolic content of 105.92 ± 0.01 mg GAE/g and total flavonoid content of 61.8 ± 0.05 mg QE/g. The lowest total phenolic content was exhibited at a condition of 300 W for 30 seconds (13.40 ± 0.08 mg GAE/g). The study concluded that microwave power showed a significant contribution to total phenolic content. It is noteworthy to highlight that microwaves could also extend shelf life by reducing microbial load in *Piper*. The study did not exhibit a significant loss of the physical structure of plant-based products.

3.5. Enzyme-Assisted Extraction

The enzyme-assisted extraction process involves enzymes breaking down the plant's cell wall to release the target compounds. Pectinase, hemicellulase, cellulase, amylase, and protease are commonly used enzymes for extraction. Plant materials with complex chemical structures can be broken down by these enzymes. In addition to isolating high-value phytochemicals, the enzymes are preferred for extracting bioactive compounds, particularly phenolic compounds. As enzyme-assisted extraction is performed at low temperatures, it is considered a mild technique. Cell walls can be disintegrated by selective ionization even at low temperatures. One of the important processes is the degradation of the plant cell wall structure, which could facilitate the release of these intracellular compounds. The effectiveness of enzyme treatments depends on the temperature, enzyme concentration, and the amount of time the enzyme is exposed to the material. The enzyme-assisted extraction process results in the extraction of high levels of phenolic compounds. By breaking and softening the structure, increasing porosity, and enhancing cell wall breakdown, the enzyme softens and breaks the structure. According to the researchers, these methods are user-friendly, efficient, and environmentally friendly. Using enzymes such as cellulase at concentrations of 0.15%, 0.12%, 0.10%, 0.08%, 0.05%, and 0.0%, Hu et al. (2020) studied surfactant.

4. COMPREHENSIVE IDENTIFICATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS FOR *PIPER* PLANTS

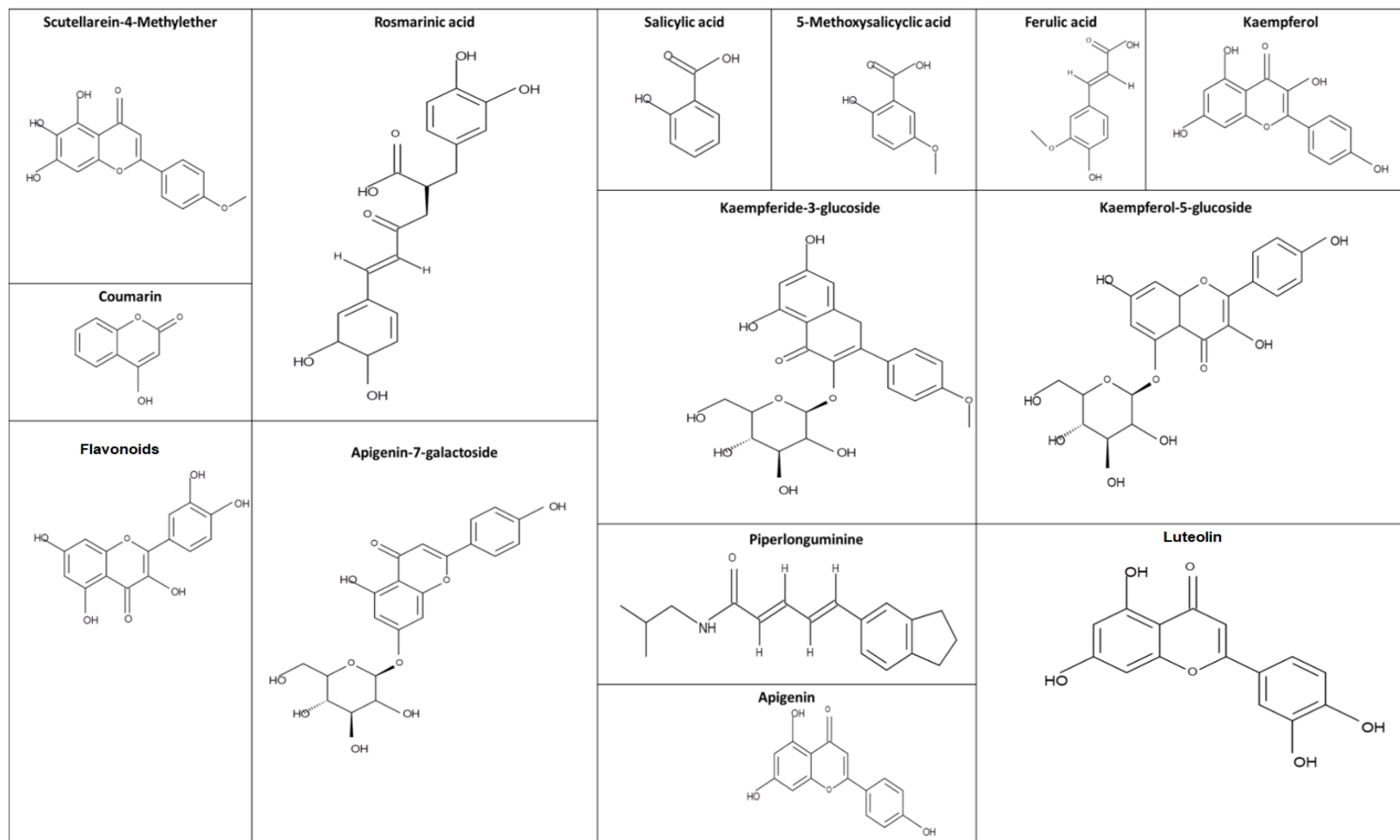
The bioactive compounds found in *Piper* plants have garnered growing interest due to their biological activity, particularly as natural products with various important applications. Recent technology, especially in integrating artificial intelligence, has improved the efficiency of scientific instruments such as chromatographic and spectrophotometric quantification. Numerous research achievements have been made in identifying and quantifying phenolic compounds, especially in *Piper* plants. Figure 2 shows the trends from 2014 to 2024 that establish a good basis for understanding the identification and quantification of *Piper*. Table 2 summarizes the extraction of *Piper* by classic and modern extraction techniques.

Table 2. Extraction of Piper from 2010 to 2025 by classic and modern extraction technique.

| Plant | Part | Sources | Extraction | Extraction parameters | Analysis | Total phenolic content | References |
|---------------------|-------|---|---|---|--|---|---|
| <i>P. betel</i> | Leaf | Mueang district, Phatthalung province, Thailand | Modified maceration method | i. Solvent: 95 % methanol and 95 % ethanol | i. Antibacterial activity ii. Minimum inhibitory concentration (MIC) and minimum of bactericidal concentration (MBC) iii. Storage time iv. Thermal stability v. Antioxidant activity vi. Total phenolic content | 130 ± 4.46 and 147.69 ± 0.03 mg GAE/g | Sonphakdi, Tani, Payaka, and Ungcharoenwiwat (2024) |
| <i>P. betel</i> | Leaf | Paan Mandi Laxmi Bazaar, Naka Hindola, Lucknow, India | Ultrasound-assisted extraction | i.Ethanol | i. DPPH ii. Extraction yield iii. Total phenolic content iv. Total flavonoid content v. Total chlorophyll content | 192 mg GAE/g, 105 mg GAE/g. | Singh et al. (2024) |
| <i>P. nigrum L.</i> | Fruit | Provence, France | ASE® extraction | i.Hexane | i.Chromatographic ii.LC-MS/MS analysis | - | Lafeuille, Brun, Lefèvre, Menezes, and Candalino (2022) |
| <i>P. nigrum L.</i> | Fruit | Malaysian pepper board (MPB). | Microwave reflux extraction | i.Distilled water | i.Taguchi optimization ii.LCMS-QToF component analysis iii.Structural characterization | - | Olalere et al. (2018) |
| <i>P. nigrum L.</i> | Fruit | Hainan, China. | Surfactant-assisted enzymatic hydrolysis technology | i.Ethanol ii.Methanol | i.Effect of cellulose enzyme amount ii.Enzyme hydrolysis time iii.Effect of the solid–liquid ratio iv.Effect of different surfactants addition v. Granularity of pepper powder | - | Yu et al. (2020) |
| <i>P. wallichii</i> | Stem | Arunachal Pradesh, India. | Solvent extraction | i.Petroleum ii.Ether iii.Chloroform iv.Ethyl v.Acetate vi.Methanol vii.Ethanol | i.DPPH ii.Total phenolic content iii.Total Flavonoid content iv.FRAP v.ABTS | 30.47 ± 1.05 to 75.37 ± 1.75 mg GAE/g DW. | Tamuly, Hazarika, Bora, and Gajurel (2014) |

| Plant | Part | Sources | Extraction | Extraction parameters | Analysis | Total phenolic content | References |
|-------------------------|-------|----------------------------------|---|---|--|---------------------------------|--|
| <i>P. longum</i> | Fruit | Mumbai, India | i.Ultrasound assisted extraction ii.Soxhlet extraction iii.Batch solvent extraction | i.Ethanol ii.Acetone iii.Hexane | i.The effect of different solvents ii. Effect of ultrasound frequency and ultrasound power iii.Effect of temperature | - | Rathod and Rathod (2014) |
| <i>P. guineense</i> | Fruit | Imo State, South Eastern Nigeria | Sequential extraction | i.Hexane ii.Chloroform iii.Ethanol iv.Methanol | i.HPLC-UV/DAD method ii.UHPLC/Q-TOF MS method iii.Bacterial strains | - | Mgbeahuruike, Fyhrquist, Vuorela, Julkunen-Tiitto, and Holm (2018) |
| <i>P. trichostachya</i> | Leaf | Karnataka, India | Microextraction | i.Hexane ii.Methanol | i.Total flavonoid content ii.Total phenolic content iii.FRAP iv.DPPH | 6.26 mg TAE/g to 13.01 mg TAE/g | Al-Khayri et al. (2022) |
| <i>P. sarmentosum</i> | Leaf | Southeast Asia | i.Maceration ii.Ultrasonic-assisted extraction iii.Microwave-assisted iv.Infusion (MAI) v.Water bath extraction | i.Methanol | i. Total phenolic content | 379 mg GAE/100 g | Ibrahim, Arifin, and Hasali (2022) |

Note: GAE, gallic acid equivalent; RE, Rutin equivalent; DW, dried weight.

Figure 1. Phenolic compounds from *Piper* plants.

Source: Arunachalam et al. (2020).

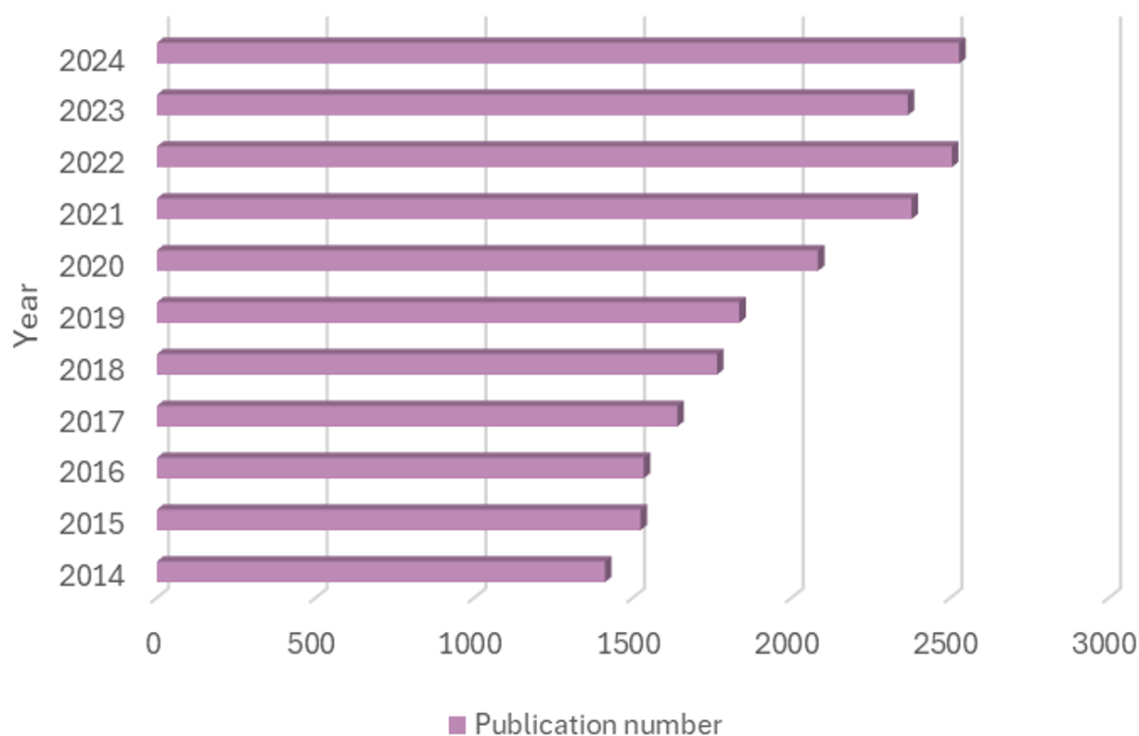


Figure 2. Research publication on *Piper* plants over the last decades (2014 to 2024).

Phenolic compounds are valuable as pharmaceutical agents, especially for inhibiting free radicals and combating cancer. Quantification methods have been developed to obtain the maximum amounts of phenolic compounds possible.

4.1. Spectrophotometric Method

Plant materials can be quantified by spectrophotometry using phenolic compounds. The measurement of phenolic compounds in various biological environments has been reported using numerous analytical approaches over the past few years. Analyses based on the spectrophotometric method are available for a variety of sample types under different sample conditions and require various sample preparations. Plant samples show similar biological activity to those measured by most of the currently available analytical techniques, especially for determining antioxidant properties. A number of studies have examined the antioxidant properties of plant samples over the past decade. *Piper* plants have been found to be potential natural antioxidant agents in these studies, providing insight into the plant. A DPPH free radical assay is the most prevalent and widely studied antioxidant assay (Pammi et al., 2024). It is becoming increasingly popular to use the spectrophotometric method due to its simplicity, speed, and cost-effectiveness.

4.2. Chromatographic Method

It is one of the most important analytical methods used to identify, separate, detect, and purify plant materials, particularly aromatic spices such as *Piper* plants. Analyses of both qualitative and quantitative nature are carried out using this method. In order to analyze phenolic compounds from *Piper* plants, many chromatographic techniques have been introduced over the years. These methods include gas chromatography, gas chromatography with Fourier transform infrared spectroscopy, and gas chromatography with flame ionization detector (Batista et al., 2023; Cucinotta et al., 2024). Table 3 shows the identification and quantification of *Piper* plants using chromatography.

Table 3. Identification and quantification of *Piper* plant using chromatography.

| <i>Piper</i> plants | <i>Piper</i> parts | Instruments | Columns | Compounds | Parameters | References |
|--|--------------------|---|---|---|---|---|
| <i>P. colubrinum</i> Link | Leaves | Liquid chromatography mass spectrometry LC-QTOF-MS/MS | Agilent Zorbax eclipse plus C18 column 4.6 mm x 100 mm; particle size of 3.5 µm | i) Apigenin ii) Kaempferol iii) Luteolin iv) Kaempferide-3-glucoside v) Apigenin-7-galactoside vii) 5-Methoxysalicylic acid viii) Kaempferol-5-glucoside ix) Slicylic acid x) Rosmarinic acid xi) Ferulic acid | i) Temperature of 40 °C. ii) Mobile phase: 10 mM iii) Solvent A : ammonium acetate Solvent B :acetonitrile iv) Flow rate:0.5 mL/min v) Gradient programme: 7.0 % B – 0 min; 45 % B – 7.0 min; 62 % B – 15 min; 85 % B – 20–25 min and 7.0 % B – 30 min). vi) Detector: 254 nm using the diode array detector. vii) Nebulizer pressure: 35 psi. | Sruthi and Zachariah (2024) |
| <i>Piper gaudichaudianum</i> Kunth | Leaves | Gas chromatography | Equity-5 poly (5% diphenyl/95% dimethylsiloxane) 30 m x 0.53 mm I.D x 5 µm | i) α-Terpinoel ii) Linalool iii) Limonene iv) β-Pinene v) α-Phellandrene vii) Camphene viii) α- thujene ix) α- Pinene | i) Temperature of 280 °C. ii) Gas pressure injector: 160 kPa, 150 kPa and 146 kPa. | Cucinotta et al. (2024) |
| <i>Piper macedoi</i> Yunck | Leaves | Gas chromatography with flame ionization detector | Fused silica capillary column 30 mm x 0.25 mm | i) α-Pinene ii) β-Pinene iii)myrcene iv) α-Phellandrene v) sylvestrene vii) Limonene viii) Linalool ix) <i>Piperitone</i> | i) Temperature: 40 °C to 240 °C to 250 °C to 280 °C ii) Flow rate: 3 mL/min | Batista et al. (2023) |
| <i>Piper guineense</i> Schumach & Thonn. | Seed | Thin layer chromatography | Silica gel column chromatography eluting with a gradient of ethyl acetate in <i>n</i> -hexane | i) <i>Piperine</i> -type amide | i) 150 mL of distilled water ii) Solvent: <i>n</i> -hexane, dichloromethane, ethyl acetate | Yimtchui et al. (2024) |
| <i>P. betle</i> | Stem root | High performance liquid chromatography | C18 column (250 mm x 4.6 mm, 5 µm) | i) <i>Piperine</i> | i) Injection volume: 20 µL ii) Temperature: Room temperature | Al-Mamun et al. (2024) |

A gas chromatography technique is beneficial for the separation of volatile compounds as well as their analysis. For the quantification and identification of both volatiles and non-volatiles in plant samples, liquid chromatography is the most suitable analytical method. Since neither derivatization nor transformation of the analytes is required in liquid chromatography, it is the most convenient technique for phenolic compounds. Chromatography columns are a major component of the separation process. A liquid-liquid chromatography column or a liquid-solid chromatography column is used for liquid chromatography. For liquid-liquid columns, the stationary phase is a liquid, while for solid-liquid columns, it is a solid. Neither the stationary phase nor the solid stationary phase is fully utilized for the liquid analyte adsorption or partition in the solid-liquid column (Avrămia, Oroian, & Oiță, 2024). The development of several chromatography methods has been observed in the last few years to achieve satisfactory separation in a reasonable amount of time. Half an hour is usually the most suitable amount of time for processing. In order to distinguish between overlapping peaks from complex compounds, sophisticated analytical tools are used. *Piper* plant materials are separated and purified by chromatography methods in many studies. For example, Cucinotta et al. (2024) used multidimensional gas chromatography to characterize eight chiral components using enantiomeric distributions. *P. gaudichaidianum* extracts were investigated by chromatography and nuclear magnetic resonance in this study.

Based on chromatography mass spectrometry, Sruthi and Zachariah (2024) revealed the relationship between volatile and non-volatile metabolite profiling in *Piper* species. Sesquiterpenes were identified as major compounds by gas chromatography in selected *Piper* species, including *P. colubrinum*. There was a predominant presence of muurolol and caryophyllene in the study. Using liquid chromatography, phenolic and alkaloid compounds were extracted from chloroform *Piper* extracts. It contains kaempferol-5-glucosides, apigenin-7-galactosides, kaempferide-3-glucosides, luteolins, kaempferide-3-glucosides, apigenins, kaempferols, and apigenins as phenolic compounds. It also contains 5-methoxysalicylic acid, rosmarinic acid, ferulic acid, and salicylic acid. *P. colubrinum* was analyzed by LC-QTOF-MS/MS at 254 nm and total ion chromatography for its non-volatile components. According to the study, *P. colubrinum* contains a wide range of high-value metabolomics, which makes it a valuable source of bioactive compounds. The most used chromatographic technique is high-performance liquid chromatography. As a technique that is capable of separating and identifying bioactive compounds, chromatography has become irreplaceable. Based on the observation between 8,586 and 8,629 min of compound identification, it was matched using 345 nm (Al-Mamun et al., 2024).

5. IN VITRO ASSESSMENT ANTIOXIDANT PROPERTIES FOR PIPER EXTRACT

Due to the presence of phenolic hydroxyl groups in their ring structures, phenolic compounds are widely recognized for their powerful ability to neutralize free radicals. Despite extensive research on radical scavenging signaling in *Piper* plants, it is important to note that an antioxidant defense system within the same species can differ significantly depending on the type of abiotic stress. In particular, the chloroplasts, peroxisomes, and mitochondria generate the radical scavenging that contributes to many varieties of properties. Ascorbic acid, butylated hydroxyanisole, butylated hydroxytoluene, and selenium are the most commonly used antioxidants.

Balekundri, Hurkadale, and Hegde (2024) conducted a thorough investigation into the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method with chromatography through the development and optimization of analytical design tools and quality assessments, such as antioxidants. The quality of the *Piper* plant is difficult to regulate due to various factors, including batch-to-batch variations in physical, chemical, and geographic characteristics. Researchers explored the connection between the *Piper* plant and its antioxidant qualities to determine the plant's potential as an antioxidant agent.

Pammi et al. (2024) conducted a study about magnesium oxide nanostructures using the *P. betle* leaf extract. The study was conducted so it could be applied to environmental and biomedical applications. According to the study, magnesium oxide with *P. betle* has antioxidant properties that can reduce stable nitrogen radicals 2,2-diphenyl-1-picrylhydrazyl. Based on their study, Al-Mamun et al. (2024) concluded that the roots and stems of the *Piper* plant,

particularly *P. chaba*, are powerful antioxidants. The study found that *Piper* plants were more recognized and more effective than synthetic drugs. According to this study, the roots exhibited strong antioxidant properties compared to the stems.

6. ANTIMICROBIAL PROPERTIES OF *PIPER* PLANT

Several studies have been conducted over the decades to find natural antibacterial remedies. It is archaic to believe that natural plants have healing properties. There has been an increase in the usage of synthetic medicines to treat diseases caused by microbiomes in recent years. The resistance of these microbiomes to medicines is also a cause for concern. Phytochemicals and secondary metabolites such as alkaloids, flavonoids, terpenoids, and tannins have been explored by microbiologists, botanists, ethnopharmacologists, and natural product chemists in search of alternative phytochemicals and secondary metabolites that could be used to treat infectious diseases caused by pathogenic bacteria. There will be fewer side effects and a lower cost associated with the new category of antibiotics. The study of secondary metabolites against bacteria has focused more on in vitro studies over the years.

The ability of plants to produce aromatic substances is almost limitless, especially phenols and their oxygen-substituted derivatives (Christaki, Bonos, Giannenas, & Florou-Paneri, 2012). Insects, microorganisms, and herbivores seek out phenols for secondary metabolites as defense mechanisms (Cowan, 1999). The antimicrobial properties of all parts of plants have been investigated. The abundance and low cost of plant leaves make it possible to investigate and develop further studies and industrialization. The potential antimicrobial properties of *Piper* leaves have been observed in numerous studies. The *Piper* species *P. betle* is one of the most popular. It has been noted that *P. betle* leaves significantly inhibit *Escherichia coli* and *Pseudomonas aeruginosa*. Additionally, it has been demonstrated that the substance inhibits Gram-positive bacteria like *Staphylococcus aureus* and *Candida albicans* (Nayaka et al., 2021). Primitive Thais have long used *P. betle* leaves to wrap and chew areca nuts and to treat black tooth stains (Sonphakdi et al., 2024). *P. retrofractum*, commonly referred to as long pepper, is another well-known piper plant leaf (Sonphakdi et al., 2024). It has been demonstrated that the main substances in *P. retrofractum*, including quinones, sterols, glycosides, tannins, flavones, and alkaloids, possess antifungal and antibacterial qualities. This paper provides a summary of the antimicrobial activity of pepper leaves over the previous few years.

A disc diffusion method is a technique used to screen extracts for antibacterial properties. Due to its widespread use and generally straightforward nature, this review focuses on this method. Some modifications were made to the disc diffusion method by Mat, Daud, Rojje, Hussain, and Rukayadi (2016). In Table 4, the antimicrobial exertion zones of pepper leaf extracts are shown.

Table 4. The inhibition zones for pepper leaves against *S. aureus*, *E. coli* and *C. albicans*.

| Pepper leaves species | Inhibition zone (mm) | | | Reference |
|-----------------------|----------------------|----------------|--------------------|--|
| | <i>S. aureus</i> | <i>E. coli</i> | <i>C. albicans</i> | |
| <i>P. betle</i> | 216.00 ± 0.40 | 232.00 ± 0.20 | No data | Sonphakdi et al. (2024). |
| <i>P. betle</i> | 18.0 ± 0.20 | 11.0 ± 0.12 | No data | Kaveti, Tan, Sarnnia, and Baig (2011) |
| <i>P. betle</i> | 18.1 ± 0.41 | 15.01 ± 0.57 | 18.13 ± 0.4 | Singh, Chauhan, Agrawal, and Mendiratta (2019) |
| <i>P. betle</i> | No data | No data | 15.00 | Sivareddy et al. (2019) |
| <i>P. refractum</i> | 13.00 | 9.00 | No data | Jamelarin and Balinado (2019) |
| <i>P. sarmentosum</i> | 11.00 | 8.00 | No data | Sanusi et al. (2017) |

In qualitative analysis, disc diffusion is often used to screen for antibacterial properties. To test the extract sensitivity afterward, dilution or microdilution methods are usually employed. It has been demonstrated that *Piper* leaves can provide natural antimicrobial properties through this investigation. A significant antibacterial effect has

been observed with the extract leaves against bacteria such as *E. coli*, *S. aureus*, and *C. albicans*. These results indicate that extracts from these leaves exhibit significant inhibitory effects against some pathogenic bacteria, highlighting their phytochemical richness and potential applications. Most plant extracts have significant flavonoid concentrations, which may contribute to their antibacterial activity (Mat et al., 2016). It is necessary to characterize and isolate the active compounds responsible for this activity, as well as to evaluate their safety and efficacy in clinical trials. *Piper* leaves are traditionally used in folk medicine, and these findings pave the way for future research aimed at developing new antibacterial treatments.

7. CHALLENGES IN UTILISATION OF PHENOLIC COMPOUNDS FROM *PIPER* PLANT FOR ITS BIOLOGICAL ACTIVITIES

It is very important to obtain phenolics from *Piper* plants. However, due to their high perishability, low dissolution rate, and high sensitivity, their application remains challenging. In order to address these specific problems, numerous studies have been conducted. Sruthi and Zachariah (2024) provide strategies for overcoming these issues. A chemical profiling of *P. columnarium* was conducted to present the properties of *Piper*. According to the study, the *Piper* plant has the potential to be incorporated into future food and nutraceutical products. The potential of AI-powered computational models is being studied to enhance the effectiveness, stability, and bioavailability of *Piper*-derived phenolic compounds in functional food applications.

Moreover, a robust and reliable extraction and sampling technique is necessary to obtain the desired phenolic compounds from plant matrices for identification and quantification using chromatography. Certain factors may cause phenolic compounds to deteriorate. In addition to intrinsic and extrinsic factors, phenolic stability can be affected by pH, temperature, moisture content, water activity, oxygen, light exposure, metallic ions, complex components, and other factors. It would be impossible to solve challenging problems in the plant bioactive compound industries without advanced food science and technology integrating basic knowledge from various disciplines such as chemistry, biology, and physics. The effectiveness of phenolic compounds depends on a clear understanding of the food's characteristics and properties. Bioactive compounds, particularly phenolic compounds, must be incorporated into food products in the future. Due to the aforementioned issue, future research will focus on improving the extraction of phenolic compounds from *Piper* plants. The challenge with all of the phytopharmaceutical properties is identifying the effective compounds and mechanisms that are responsible for their action, according to Muthusamy et al. (2024)

The lack of scientific data and awareness of the toxicology and pharmaceutical properties of phenolics from *Piper* plants is another challenge. This may be due to the fact that this plant is considered edible and safe for consumption. According to a study conducted by Yimitchui et al. (2024), the Vero cell line is critical for evaluating the cytotoxicity of non-tumorigenic cells. *Piper* compounds were found to be weakly toxic (CC₅₀ 200 g/mL) and non-toxic (CC₅₀ > 200 g/mL) to Vero cells. With the agreement of Pothipongsa et al. (2024), the *Piper* plant does not exhibit any cytotoxic potential (Pothipongsa et al., 2024). To diversify the potential of *Piper* plant phenolic compounds, extensive toxicology studies are needed.

8. POTENTIAL OF *PIPER* PLANT FOR FOOD APPLICATION

Since *Piper* plants exhibit a variety of biological activities, future research opportunities can be discussed to address current food applications and products derived from *Piper* plants. For instance, to increase product safety and shelf life. Environmental factors like oxidation and pathogenic microorganisms such as mold, yeast, and bacteria are the main causes of food poisoning. These factors lead to food degradation and contamination, which can be detrimental to health. Food becomes unfit for consumption when it deteriorates because of changes in its overall quality, texture, and appearance. The growth and activity of microorganisms typically facilitate this process, changing the product's original properties and causing foodborne illnesses (Tran, Nguyen, Nguyen, Do, & Le, 2023). Natural extracts help

to prolong the shelf life of food products and prevent microbial growth. They are also becoming increasingly popular substitutes for synthetic chemical preservatives. One such natural preservative, betel leaves (*Piper siriboa* L.), has been documented in the literature as a viable option, and the bioactive components found in betel leaves include polyphenols, flavonoids, alkaloids, eugenol, carvacrol, and chavicol. The Betel leaf extracts and essential oils are recognized for their powerful antibacterial, antifungal, anti-inflammatory, and antioxidant functions, which make them suitable for many applications, for example, food preservation. Recent research shows that some of the compounds found in betel leaves inhibit the growth of bacteria, including drug-resistant bacteria, and fungi that cause serious diseases to consumers. The bacteria involved include both gram-positive and gram-negative bacteria (Tran et al., 2023). Betel leaf is from the *Piperaceae* family of plants and is distributed primarily in Asian countries, including India, China, Indonesia, Malaysia, and Vietnam. Betel leaves are an excellent source of bioactive compounds, including phytol, 4-chromanol, allylpyrocatechols, eugenol, hydroxychavicol, chavicol, chavibetol, and carvacrol. These compounds can be categorized as extracts or monoterpenes, phenylpropanoids, aldehydes, and sesquiterpenes, many of which are largely found in essential oils. These compounds can be extracted through various methods such as maceration, Soxhlet extraction, solid-liquid extraction, ultrasound extraction, microwave extraction, steam distillation, and supercritical fluid extraction. Hydroxychavicol, eugenol, chavibetol, and chavicol are safe and non-toxic to be used as food additives. However, the addition of slaked lime, areca, and tobacco to betel leaves has been causing severe health risks such as cancers, submucosal fibrosis, and damage to the esophagus. Therefore, for all intents and purposes, betel leaf extracts and essential oils are safe when consumed as food additives, as opposed to betel leaves with the other substances (Madhumita, Guha, & Nag, 2020). Recently, AI-driven formulation strategies are being investigated to enhance the product stability and usefulness of phenolic compounds from Piper plants.

8.1. Food Product: Emulsion

Bioactive substances such as *piperine*, essential oils, and phenolics are abundant in *P. betel* extracts that have been used in food applications. They help to improve their usefulness and health advantages. These extracts can be added to water-in-oil or oil-in-water emulsions as natural antimicrobials, antioxidants, and bioavailability enhancers. Essential oils from *Piper* species not only function as natural surfactants but also help to reduce interfacial tension and increase the physical stability of emulsions. One important alkaloid, which is piperine, can improve the bioavailability of fat-soluble vitamins and nutrients when added to sauces, drinks, or salad dressings. Furthermore, the solubility, stability, and controlled release of *Piper* extracts are enhanced by encapsulation techniques such as nanoemulsion or microemulsion formulations. Emulsions with *P. betel* extracts are a useful addition to food formulations because they provide functional health benefits in addition to enhanced shelf life and sensory qualities (Singh et al., 2024).

8.2. Food Product: Fortified Food

Piper extracts, which are rich in bioactive substances like *piperine*, flavonoids, essential oils, and phenolics, can be added to a variety of foods to help improve their nutritional value and functional qualities. To guarantee optimal bioactivity, these extracts are usually made using solvent extraction, supercritical CO₂ extraction, or ultrasound-assisted extraction (UAE). They can be incorporated into food systems in several ways, such as powders, emulsions, or encapsulated goods, to ensure stability and controlled release. *Piperine*-fortified foods such as beverages, snacks, and supplements can enhance the bioavailability of vitamins and minerals. Flavonoids and phenolics, which possess antioxidant properties, as well as essential oils, which have antimicrobial properties, can all help preserve food naturally. In addition to their health benefits, *piper* extracts have sensory qualities like flavor and aroma, making them ideal for seasoning blends, health drinks, and functional snacks (Tran et al., 2025).

8.3. Food Product: Food Stabiliser

Extracts from *P. Betel* can boost the stability, functionality, and nutritional value of food products, especially those containing essential oils, phenolic compounds, and *Piperine*. The use of these extracts can improve the physical stability of emulsions and avoid phase separation in food products such as sauces, dressings, and beverages by reducing interfacial tension. In addition, the phenolics and *Piperine* can help protect food products against oxidative degradation because of their properties as antioxidants. Essential oils from *Piper* help support food safety by inhibiting the growth of spoilage microorganisms. Furthermore, these extracts can help enhance the sensory properties of food products, such as flavor and aroma. Extracting the *Piperine* and related bioactive compounds from plant materials can be done using several extraction methods involving solvents or supercritical CO₂ to maintain the bioactive compounds. Additionally, the extraction can be complemented by encapsulation techniques, such as microencapsulation or nanoencapsulation, to improve the stability and functionality of the bioactive compounds as a food ingredient. In general, the stabilization of food formulations using Piper plants (and their extracts) will promote a natural source of stabilizers, and they can provide a multifunctional ingredient as a replacement for synthetic and chemically produced food additives (Magdy et al., 2024).

8.4. Food Product: Beverages

Plant extracts such as the *P. betel* and *P. nigrum* are used in beverage products to enhance flavor, shelf life, and offer health benefits. The functional properties of piper plant extracts are related to the bioactive compounds such as *Piperine*, eugenol, and phenolics. The natural antioxidants in *P. betel* extracts allow for shelf-stable beverages by helping to counteract oxidative stress. Antimicrobial properties can inhibit spoilage microorganisms and improve the safety and stability of the beverages. *P. betel* extracts also enhance the sensory profile in beverages with their spicy and warming flavors. The bioactive compounds have anti-inflammatory and digestive properties that meet consumer expectations for functional beverages. *P. betel* extracts can be obtained by several solvent extraction methods, steam distillation, or supercritical fluid extraction. The extraction methods for beverage production can alter the functional properties of the beverage product while including the concentration and activation of functional constituents (Pothipongsa et al., 2024).

8.5. Food Product: Edible Food Packaging

Extracts from *P. betle* have been successfully added to edible food packaging materials to improve their antioxidant and antimicrobial qualities. In one study, flaxseed gum and betel leaf extract were combined to make composite edible films. The films' shelf life was increased by the addition of the betel leaf extract, which also makes them stronger, water vapor-permeable, and antimicrobial. Likewise, chitosan-based films that were infused with *P. betel* extract demonstrated increased antibacterial activity against foodborne pathogens. Researchers discovered that the *P. betel* extracts could be utilized to create active, biodegradable packaging solutions that support food safety and preservation (Arunachalam et al., 2020).

8.6. Food Product: Food Ingredient

Due to their great concentration of bioactive compounds including phenolics, flavonoids, and essential oils, *P. betle* extracts have attracted interest for their antimicrobial and antioxidant effects. These extracts, when added to food products, help to increase shelf life, lower bacterial contamination, and improve the safety of the food product. Betel leaf extract has been found to greatly reduce spoilage microorganisms, providing longer storage without compromising quality. For example, the traditional Malaysian chili paste (chili bo). The *P. betle* extracts are usually obtained by using solvent extraction techniques, such as ethanol extraction. The extracts are meant to maximize the retention of active compounds. *P. betle* natural preservatives support clean-label trends by providing customers with a healthy and chemical-free substitute for synthetic additions (Sonphakdi et al., 2024).

8.7. Food Product: Complex Food Models

P. betle extracts have strong antimicrobial and antioxidant properties, specifically due to bioactive compounds like phenolics and flavonoids. Food safety and quality can be improved by adding these extracts to biocompatible, multifunctional food modules, such as edible films or coatings. Research has demonstrated that incorporating betel leaf extract counteracts the decline of mechanical strength, barrier properties, and antibacterial properties of sago starch-based films, thereby providing a means to extend the films' shelf life. These bioactive compounds are then extracted using solvents to ensure their ability to retain functional efficacy. By combining the *P. betle* extracts within advanced food systems, manufacturers will be able to deliver food systems that are natural, sustainable, functional, chemical-free, and clean-label ecosystems.

8.8. Food Product: Plant Protein

The possibility of *P. betel* extracts improving the functional characteristics of plant-based protein foods has been emphasized. Including the extracts in the plant protein formulations can enhance antimicrobial and antioxidant action, thereby extending shelf life and increasing safety. The enhanced antioxidant and antibacterial properties of dechlorophyllized betel leaf ethanolic extract suggest that it might be used to preserve food. Ethanol, usually used as a solvent, is extracted; then, dechlorophyllization techniques, including sedimentation, help to lower the chlorophyll content. Including *P. betel* extracts in plant protein-based foods will help satisfy consumer tastes for clean-label ingredients and increase product stability (Lafeuille et al., 2022).

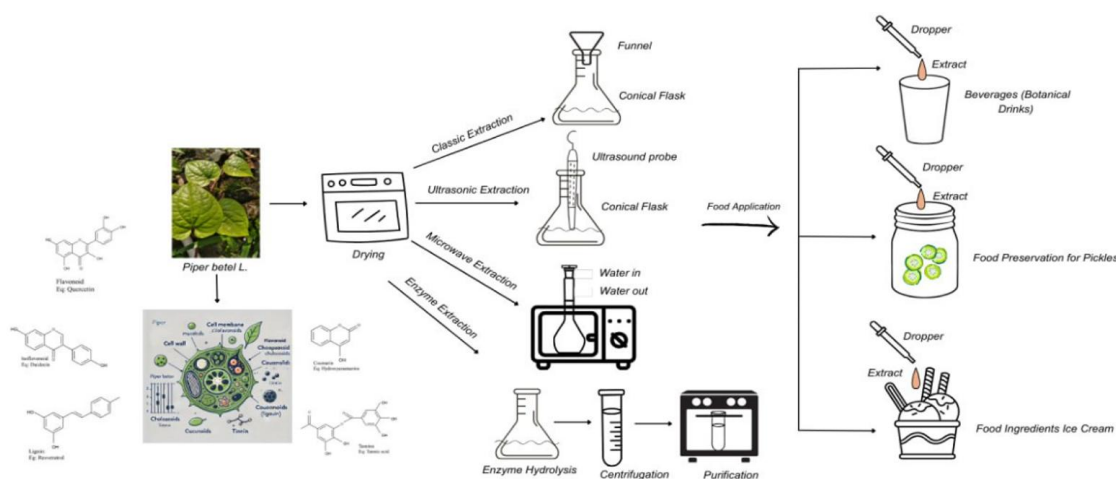


Figure 3. Overview of the extraction technique and their various applications in the food applications.

Figure 3 illustrates the possible extraction techniques of the Piper plant. The Piper is subjected to a drying process and proceeds with various extraction techniques such as classic extraction, ultrasonic extraction, and enzyme extraction. The Piper extract is subsequently used directly in food applications such as beverages, food ingredients, and as a food preservative.

9. CONCLUSION

The secondary metabolites of *Piper* species exhibit various phytopharmaceutical properties, including antioxidant and antimicrobial properties. To optimize extraction efficiency, advanced technology is used in addition to traditional extraction methods to enhance solvent solubility and increase the extraction efficiency of phenolic compounds. Spectroscopy and chromatography require efficient extraction techniques to successfully isolate phenolic compounds from plant matrices for identification and quantitation of phenolic compounds from *Piper* plants. Despite its rich nutritional and phytochemical profile, the *Piper* plant has remained largely unutilized. Due to the lack of awareness about its health benefits and the presence of bioactive compounds, its application to food is limited. The future of

research will also require scaling up the process and studying its environmental impact. The integration of AI into the extraction of *Piper* species represents a transformative advancement in natural product research. AI-powered technologies, including machine learning, automation, and real-time monitoring, significantly improve extraction efficiency, maintain bioactive compound integrity, and encourage sustainable practices. As AI continues to evolve, its role in phytochemical extraction will expand, paving the way for more efficient, eco-friendly, and data-driven botanical research.

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