WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 16, 689-707. Research Article ISSN 2277–7105

PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACTS OF INDIGENOUS MEDICINAL PLANT *PIPER CHABA*

Md. Babul Hossain[1], Md. Abdul Mannan[2]*, Sumon Kanti Datta[3], Md. Saiful Islam[4]

[1]Professor, Prafulla Sinha Ayurvedic Medical College, Parnanduali, Magura Sadar, Magura.

[2]Associate Professor, Department of Unani Medicine, Faculty of Unani and Ayurvedic Medicine, Hamdard University Bangladesh.

[3]Research Officer, Center for Natural Remedies, Rajarbag, Dhaka.

[4]Assistant Professor, Rawshan Jahan Eastern Medical College & Hospital (Unani), South Maguri, Dattapara, Lakshmipur -3706.

Article Received on 13 July 2026,
Article Revised on 03 August 2026,
Article Published on 16 August 2026,

https://doi.org/10.5281/zenodo.21915612

***Corresponding Author**

Md. Abdul Mannan

Associate Professor, Department of Unani Medicine, Faculty of Unani and Ayurvedic Medicine, Hamdard University Bangladesh.

How to cite this Article: Md. Babul Hossain[1], Md. Abdul Mannan[2]*, Sumon Kanti Datta[3], Md. Saiful Islam[4] (2026). Phytochemical Screening Of Methanolic Extracts Of Indigenous Medicinal Plant Piper Chaba. World Journal of Pharmaceutical Research, 15(16), 689–707.

ABSTRACT

Medicinal plants have served as an important source of therapeutic agents for centuries and continue to play a significant role in the discovery of novel drugs. The increasing prevalence of antimicrobial resistance, chronic diseases, and the adverse effects associated with synthetic drugs has renewed interest in medicinal plants as safer and more affordable alternatives. *Piper chaba*, an indigenous medicinal plant belonging to the family Piperaceae, is widely distributed in South and Southeast Asia and has been traditionally used for the treatment of cough, asthma, fever, digestive disorders, rheumatism, inflammation, and various microbial infections. Despite its widespread ethnomedicinal use, scientific validation of its phytochemical constituents remains limited. Therefore, the present study aimed to qualitatively screen the phytochemical constituents present in the methanolic extract of *Piper chaba* using standard phytochemical screening methods. Fresh plant material of *Piper chaba* was collected, authenticated, shade-dried, and powdered before extraction with methanol. The methanolic extract was subjected to preliminary qualitative phytochemical screening following established laboratory protocols. Standard chemical tests were performed to detect the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols,

phenols, tannins, flavonoids, proteins, and amino acids. Alkaloids were identified using Mayer's, Wagner's, Dragendorff's, and Hager's tests. Carbohydrates were evaluated using Molisch's, Benedict's, and Fehling's tests. Other phytochemicals were detected using specific qualitative assays, including the Modified Borntrager's test, Legal's test, Froth test, Foam test, Salkowski's test, Liebermann–Burchard's test, Ferric chloride test, Gelatin test, Alkaline reagent test, Lead acetate test, Xanthoproteic test, and Ninhydrin test. The qualitative analysis revealed the presence of several biologically active secondary metabolites in the methanolic extract of *Piper chaba*. Alkaloids were detected by all four alkaloid tests, indicating their abundance in the extract. Glycosides, phytosterols, phenolic compounds, tannins, flavonoids, and proteins also produced positive results in their respective tests. Among the carbohydrate tests, Benedict's test was positive, indicating the presence of reducing sugars, whereas Molisch's and Fehling's tests were negative. The extract did not show the presence of saponins, as both the Froth and Foam tests were negative. Similarly, the Ninhydrin test was negative, suggesting the absence of free amino acids, although proteins were detected by the Xanthoproteic test. The presence of these phytochemicals suggests that *Piper chaba* possesses significant pharmacological potential. Alkaloids, flavonoids, phenolic compounds, and tannins are known for their antioxidant, antimicrobial, anti-inflammatory, and anticancer properties, while phytosterols and glycosides contribute to various therapeutic effects. The findings of this study support the traditional medicinal use of *Piper chaba* and indicate that methanol is an effective solvent for extracting its bioactive constituents. This preliminary phytochemical investigation provides a scientific basis for further quantitative phytochemical analysis, isolation and characterization of active compounds, and pharmacological evaluation to establish the therapeutic efficacy of *Piper chaba*. The study contributes to the growing body of knowledge on indigenous medicinal plants and highlights the importance of conserving and scientifically exploring plant resources for future drug development.

KEYWORDS: *Piper chaba*, phytochemical screening, methanolic extract, medicinal plant, alkaloids, flavonoids, phenolic compounds, glycosides, phytosterols.

1.1. INTRODUCTION

Medicinal plants have been used as natural remedies for the prevention and treatment of diseases since ancient times. They remain an important component of traditional healthcare systems and continue to contribute significantly to modern medicine. According to the World Health Organization (WHO), approximately 80% of the population in developing countries

depends partly or entirely on medicinal plants for primary healthcare (WHO, 2013). The therapeutic properties of medicinal plants are mainly attributed to the presence of biologically active secondary metabolites, commonly known as phytochemicals, which exhibit a wide range of pharmacological activities including antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, and cardioprotective effects (Harborne, 1998; Evans, 2009). Consequently, scientific investigation of medicinal plants has become increasingly important for identifying novel bioactive compounds that may contribute to the development of safe and effective therapeutic agents.

Phytochemicals are naturally occurring chemical compounds synthesized by plants as part of their normal metabolic processes. These compounds are generally classified into several major groups, including alkaloids, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, steroids, saponins, and essential oils. Although these metabolites are not directly involved in the primary growth and development of plants, they play vital roles in defence against pathogens, herbivores, and environmental stress (Harborne, 1998). Numerous studies have demonstrated that phytochemicals possess significant biological activities that contribute to disease prevention and treatment in humans (Pandey & Rizvi, 2009). Therefore, phytochemical screening is an essential preliminary step in medicinal plant research because it provides valuable information regarding the chemical constituents responsible for therapeutic effects.

The genus *Piper* belongs to the family Piperaceae and comprises more than 2,000 species distributed throughout tropical and subtropical regions of the world (Parmar et al., 1997). Several *Piper* species have been extensively used in traditional medicine because of their diverse pharmacological properties. Plants belonging to this genus contain numerous biologically active compounds, including alkaloids, amides, lignans, flavonoids, phenolic compounds, terpenoids, and essential oils, many of which have demonstrated antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, insecticidal, and anticancer activities (Dyer & Palmer, 2004; Salehi et al., 2019). The medicinal importance of *Piper* species has attracted considerable scientific attention, leading to increased investigations of their phytochemical composition and biological activities.

Piper chaba Hunter, commonly known as Java long pepper or Choi Jhal in Bangladesh, is an indigenous medicinal plant widely distributed throughout Bangladesh, India, Nepal, Myanmar, Thailand, and other Southeast Asian countries (Rahman, 2013). The plant is a

perennial woody climber characterized by aromatic stems, leaves, and fruits that are commonly used as spices and herbal medicines. Different parts of *Piper chaba*, particularly the fruits and roots, have been traditionally used in Ayurvedic, Unani, and folk medicine for the treatment of asthma, cough, bronchitis, indigestion, diarrhoea, dysentery, fever, rheumatism, toothache, and inflammatory disorders (Khare, 2007). In rural communities, preparations of *Piper chaba* are also used to stimulate digestion, improve appetite, relieve abdominal pain, and treat microbial infections, reflecting its broad therapeutic applications in traditional healthcare systems.

The medicinal value of *Piper chaba* is primarily associated with its rich phytochemical composition. Previous investigations have reported that the plant contains alkaloids, flavonoids, tannins, phenolic compounds, glycosides, steroids, lignans, volatile oils, and amide compounds such as piperine and chavicine, which are responsible for many of its biological activities (Parmar et al., 1997; Salehi et al., 2019). Piperine, one of the major bioactive alkaloids present in *Piper* species, has attracted considerable interest because of its antioxidant, antimicrobial, anti-inflammatory, anticancer, immunomodulatory, and bioavailability-enhancing properties (Srinivasan, 2007). The presence of these phytochemicals suggests that *Piper chaba* may serve as a valuable source of natural therapeutic agents.

In recent years, there has been increasing global concern regarding the emergence of antimicrobial resistance, chronic inflammatory diseases, diabetes mellitus, cardiovascular disorders, and cancer. Although synthetic drugs remain the cornerstone of modern medicine, prolonged use may result in adverse side effects, drug resistance, toxicity, and high treatment costs (Newman & Cragg, 2020). Consequently, researchers have increasingly focused on medicinal plants as alternative or complementary sources of bioactive compounds capable of addressing these healthcare challenges. Natural products continue to play a vital role in pharmaceutical research, with a substantial proportion of currently approved drugs being derived directly or indirectly from plant sources (Newman & Cragg, 2020). Therefore, the scientific validation of traditionally used medicinal plants has become a priority in natural product research.

Methanol is among the most widely used solvents for phytochemical extraction because of its ability to dissolve a broad spectrum of polar and moderately non-polar compounds, including phenolics, flavonoids, alkaloids, tannins, and glycosides (Azwanida, 2015). Methanolic

extraction has been shown to yield higher concentrations of biologically active constituents compared with many other organic solvents, making it particularly suitable for preliminary phytochemical investigations. Qualitative phytochemical screening using standard chemical methods provides rapid and reliable identification of different classes of secondary metabolites before undertaking quantitative analysis or isolation of individual compounds (Harborne, 1998).

Despite the widespread traditional use of *Piper chaba*, relatively few studies have comprehensively investigated its phytochemical profile using standardized laboratory methods. Most available research has focused on pharmacological activities rather than the systematic identification of its chemical constituents. Therefore, further phytochemical evaluation is required to provide scientific evidence supporting its ethnomedicinal applications and to identify bioactive compounds that may be responsible for its therapeutic effects. Such information may contribute to future pharmacological, toxicological, and pharmaceutical studies aimed at developing plant-based medicines.

The present study was therefore undertaken to perform a qualitative phytochemical screening of the methanolic extract of the indigenous medicinal plant *Piper chaba*. Standard phytochemical tests were employed to detect the presence or absence of major classes of secondary metabolites, including alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds, tannins, flavonoids, proteins, and amino acids. The findings of this investigation will provide baseline scientific information regarding the phytochemical composition of *Piper chaba* and contribute to the growing body of knowledge on medicinal plants. Furthermore, the results may serve as a foundation for future quantitative phytochemical analyses, isolation and characterization of active constituents, and pharmacological studies exploring the therapeutic potential of this indigenous medicinal species.

1.2. PLANT PROFILE OF *PIPER CHABA*

Piper chaba is an economically and medicinally important climbing plant belonging to the family Piperaceae. It is widely distributed throughout South and Southeast Asia, particularly in Bangladesh, India, Thailand, and Myanmar, where it is valued as both a spice and a traditional medicinal plant. The plant contains numerous bioactive compounds, including piperine, piplartine, and chabamides, which exhibit antimicrobial, antioxidant, anti-inflammatory, anticancer, and hepatoprotective activities. Besides its pharmacological

significance, *P. chaba* contributes to local livelihoods through its culinary and commercial value. This article presents an overview of the taxonomy, morphology, habitat, distribution, phytochemistry, traditional uses, and ecological importance of *P. chaba*, highlighting its potential for future pharmaceutical and agricultural research.

Medicinal plants continue to play an essential role in healthcare systems worldwide, particularly in developing countries where traditional medicine remains an important source of primary healthcare. Among these plants, *Piper chaba* Hunter has attracted considerable scientific interest because of its culinary value and broad spectrum of pharmacological activities (Islam et al., 2020). Commonly known as Choi Jhal in Bangladesh and Chavya in India, the plant is widely used as a spice and herbal remedy for treating digestive disorders, respiratory diseases, inflammation, and microbial infections (Islam et al., 2020). Recent phytochemical and pharmacological studies have validated many of these traditional applications, making *P. chaba* a promising source of natural bioactive compounds.

The species belongs to the diverse genus *Piper*, which comprises over 1,000 species distributed mainly in tropical and subtropical regions (Salehi et al., 2019). Some taxonomic authorities currently treat *Piper chaba* as synonymous with *Piper retrofractum*, reflecting ongoing revisions within the genus (POWO, 2024). *Piper chaba* is a perennial woody climbing vine that grows by twining around trees and other supports. The stem is slender, cylindrical, green when young, and gradually becomes woody with age. Adventitious roots emerge from the nodes, enabling effective climbing. The leaves are simple, alternate, ovate to elliptic, glossy green, and aromatic when crushed. Leaf margins are entire with pointed apices. The plant produces small, inconspicuous flowers arranged in elongated spikes. The flowers are generally unisexual and bloom during the rainy season. The fruit consists of numerous fleshy berries compactly arranged on cylindrical spikes. Immature fruits are green and become red upon ripening before turning dark brown or black when dried. The fruits, stems, and roots possess a characteristic pungent aroma due to their rich alkaloid content (National Center for Biotechnology Information (NCBI, 2023).

Figure 2.1: *Piper chaba* plant.

Figure 2.2: *Piper chaba* roots.

The taxonomic classification of *Piper chaba* is as follows.

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Piperales

Family: Piperaceae

Genus: *Piper*

Species: *Piper chaba*

Piper chaba is native to South and Southeast Asia and is commonly found in Bangladesh, India, Nepal, Myanmar, Thailand, Malaysia, Indonesia, and Sri Lanka (POWO, 2024). In Bangladesh, cultivation is concentrated in the southwestern districts, particularly Khulna, Jashore, Satkhira, and Bagerhat, where it is extensively cultivated for culinary purposes (Islam et al., 2020). The species thrives in tropical and subtropical climates with high humidity. It prefers shaded environments, fertile loamy soils rich in organic matter, and well-drained conditions. Naturally, it occurs along forest margins and under forest canopies where adequate moisture is available throughout the year (NCBI, 2023).

Phytochemical investigations have revealed that *Piper chaba* contains a diverse range of biologically active compounds. Major constituents include piperine, piplartine (piperlongumine), chabamides, retrofractamides, sesamin, pipernonaline, dehydropipernonaline, essential oils, lignans, alkaloids, flavonoids, and phenolic compounds (Islam et al., 2020). These compounds are largely responsible for the plant's pungency and medicinal properties (Salehi et al., 2019). For centuries, *Piper chaba* has been used in

traditional Ayurvedic and folk medicine. Different plant parts are employed to treat indigestion, diarrhea, dyspepsia, asthma, bronchitis, rheumatism, fever, and parasitic infections. Modern pharmacological studies have demonstrated antimicrobial, antioxidant, anti-inflammatory, analgesic, antidiabetic, hepatoprotective, gastroprotective, immunomodulatory, antimalarial, and anticancer activities of its extracts and isolated compounds (Islam et al., 2020).

In Bangladesh, the stem is widely used as a premium spice in beef, mutton, and fish curries, especially in the Khulna region, where Choi Jhal has become an important regional culinary ingredient. Increasing consumer demand has significantly enhanced its commercial value, making it an important cash crop for small-scale farmers (Islam et al., 2020). As a climbing understory species, *Piper chaba* contributes to forest biodiversity by providing habitat and food resources for insects and other organisms. Sustainable cultivation of the species can reduce harvesting pressure on wild populations while supporting biodiversity conservation and rural livelihoods. Although the species is not currently considered globally threatened, habitat degradation and excessive collection from natural forests may affect local populations (POWO, 2024). *Piper chaba* Hunter represents an important medicinal and economic plant with considerable ethnobotanical significance. Its rich phytochemical composition supports numerous pharmacological activities that validate its traditional medicinal applications (Islam et al., 2020; Salehi et al., 2019). Furthermore, its commercial importance as a culinary spice provides substantial economic opportunities for local communities. Continued research focusing on phytochemistry, pharmacology, conservation, and sustainable cultivation will further enhance the utilization and conservation of this valuable plant species.

2.0. AIM AND OBJECTIVES OF THE STUDY

2.1. General Objective

The primary aim of this study is to qualitatively evaluate the phytochemical constituents present in the methanolic extract of the indigenous medicinal plant *Piper chaba*.

2.2. Specific Objectives

1. To prepare a methanolic extract of *Piper chaba* using standard extraction procedures.
2. To perform qualitative phytochemical screening of the methanolic extract.
3. To determine the presence or absence of major phytochemical groups, including alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, terpenoids, steroids, carbohydrates, and proteins.

4. To provide scientific evidence supporting the traditional medicinal use of *Piper chaba*.

3.0. METHODOLOGY

3.1. Study Design

The present study employed an experimental laboratory-based design to qualitatively investigate the phytochemical constituents of the methanolic extract of the indigenous medicinal plant *Piper chaba*. Standard phytochemical screening procedures were used to identify the presence or absence of major classes of bioactive compounds, including alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins, and amino acids. These qualitative methods are widely accepted for preliminary phytochemical investigations because they provide rapid, simple, and reliable identification of plant secondary metabolites (Harborne, 1998).

3.2. Collection and Identification of Plant Material

Fresh samples of *Piper chaba* were collected from a local area in Birat, Batiaghata, Khulna district**,** Bangladesh during month of January. Healthy and disease-free plant materials were selected to ensure sample quality. The collected specimens were washed thoroughly with running tap water to remove dust and other contaminants before being rinsed with distilled water. Botanical identification and authentication of the plant were performed using standard taxonomic procedures by a qualified botanist from Hamdard University Bangladesh. After identification, the plant materials were shade-dried at room temperature for approximately two weeks to preserve thermolabile phytochemical constituents (Evans, 2009).

3.3. Preparation of Plant Extract

The dried plant materials were ground into a fine powder using a clean electric grinder. Approximately 100 g of the powdered sample was soaked in 500 mL of analytical-grade methanol and kept in a tightly closed glass container for 72 hours with occasional shaking to facilitate extraction. The extract was filtered first through clean muslin cloth and subsequently through Whatman No. 1 filter paper to remove insoluble materials. The filtrate was concentrated using a rotary evaporator under reduced pressure at a controlled temperature below 45°C to obtain the crude methanolic extract. The concentrated extract was transferred into an airtight glass container and stored at 4°C until further phytochemical analysis (Azwanida, 2015).

3.4. Qualitative Phytochemical Screening

Preliminary phytochemical screening of the methanolic extract was performed using standard qualitative methods described by Harborne (1998) and Trease and Evans (2009). Alkaloids were detected using Mayer's, Wagner's, Dragendorff's, and Hager's tests. Carbohydrates were identified using Molisch's, Benedict's, and Fehling's tests. Glycosides were determined using Modified Borntrager's and Legal's tests. Froth and Foam tests were performed for the detection of saponins, while phytosterols were identified using Salkowski's and Liebermann–Burchard's tests. Phenolic compounds and tannins were detected using Ferric chloride and Gelatin tests, respectively. Flavonoids were identified by Alkaline reagent and Lead acetate tests. Proteins and amino acids were examined using Xanthoproteic and Ninhydrin tests, respectively. The development of characteristic colour changes or precipitates was recorded as positive reactions according to established laboratory protocols (Harborne, 1998; Evans, 2009).

3.5. Recording and Interpretation of Results

The observations obtained from each phytochemical test were carefully recorded and tabulated. The presence of characteristic colour changes or precipitate formation indicated a positive (+) reaction, whereas the absence of such changes indicated a negative (–) reaction. The experimental findings were summarized in tables to facilitate interpretation and comparison with previously published studies. Since the study focused on qualitative phytochemical analysis, no statistical analysis was performed.

4.0. RESULTS AND DISCUSSION

4.1. Results of Phytochemical Screening of the Methanolic Extract of *Piper chaba*

The methanolic extract of *Piper chaba* was subjected to qualitative phytochemical screening using standard laboratory procedures to identify the major classes of bioactive constituents. The results revealed the presence of several important secondary metabolites, including alkaloids, glycosides, phytosterols, phenolic compounds, tannins, flavonoids, proteins, and reducing sugars. However, saponins and free amino acids were not detected in the extract. The phytochemical profile obtained in the present study is summarized in Table 4.1.

Table 4.1: Qualitative Phytochemical Screening of the Methanolic Extract of *Piper chaba*.

Phytochemical	Test	Observation
Alkaloids	Mayer's test	Positive (+)
	Wagner's test	Positive (+)
	Dragendorff's test	Positive (+)
	Hager's test	Positive (+)
Carbohydrates	Molisch's test	Negative (−)
	Benedict's test	Positive (+)
	Fehling's test	Positive (+)
Glycosides	Modified Borntrager's test	Positive (+)
	Legal's test	Positive (+)
Saponins	Froth test	Negative (−)
	Foam test	Negative (−)
Phytosterols	Salkowski's test	Positive (+)
	Liebermann–Burchard's test	Positive (+)
Phenols	Ferric chloride test	Positive (+)
Tannins	Gelatin test	Positive (+)
Flavonoids	Alkaline reagent test	Positive (+)
	Lead acetate test	Positive (+)
Proteins	Xanthoproteic test	Positive (+)
Amino acids	Ninhydrin test	Negative (−)

The phytochemical analysis confirmed the presence of alkaloids in all four alkaloid detection tests, indicating that alkaloids are among the major constituents of the methanolic extract. Glycosides were also detected by both Modified Borntrager's and Legal's tests, while phytosterols were confirmed through positive Salkowski's and Liebermann–Burchard's reactions.

The ferric chloride test demonstrated the presence of phenolic compounds, and the gelatin test confirmed the occurrence of tannins. Flavonoids were successfully detected by both alkaline reagent and lead acetate tests. Among the carbohydrate assays, only Benedict's test yielded a positive result, suggesting the presence of reducing sugars, whereas Molisch's and Fehling's tests were negative. The extract did not contain detectable levels of saponins or free amino acids, as indicated by negative froth, foam, and ninhydrin tests. Protein was detected by the xanthoproteic test.

Overall, the methanolic extract of *Piper chaba* demonstrated a rich phytochemical composition consisting mainly of alkaloids, flavonoids, phenols, tannins, glycosides, phytosterols, proteins, and reducing sugars.

Figure 4.1. Mayer's test

Figure 4.2. Wagner's test

Figure 4.3. Dragendorff's test

Figure 4.3. Hager's test

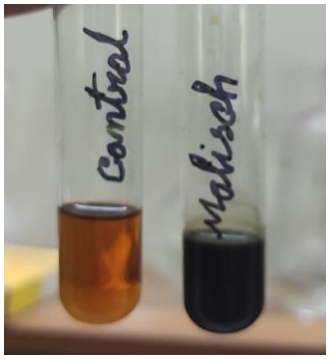

Figure 4.5. Molisch's test

Figure 4.6. Benedict's test

Figure 4.7. Fehling's test

Figure 4.8. Modified Borntrager's test

Figure 4.9. Legal's test

Figure 4.10. Froth test

Figure 4.11. Foam test

Figure 4.12 Salkowski's test

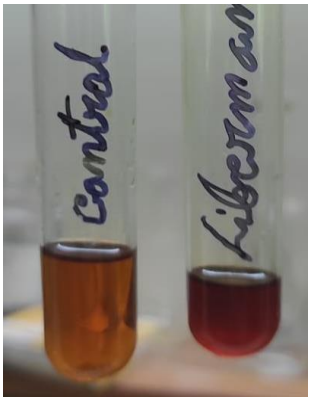

Figure 4.13. Liebermann–Burchard's test

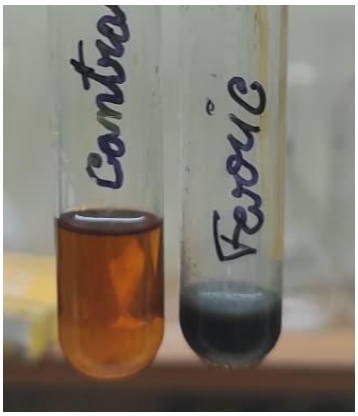

Figure 4.14. Ferric chloride test

Figure 4.15. Gelatin test

Figure 4.16. Alkaline reagent test

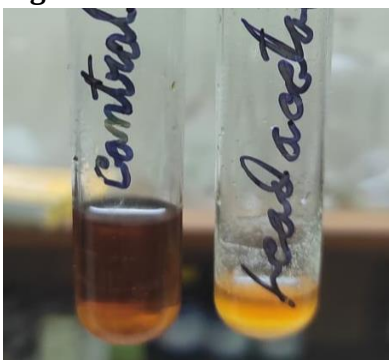

Figure 4.17. Lead acetate test

Figure 4.18. Xanthoproteic test

Figure 4.19. Ninhydrin test

4.2. DISCUSSION

Medicinal plants contain numerous secondary metabolites that are responsible for their therapeutic properties. The present study qualitatively evaluated the phytochemical constituents of the methanolic extract of *Piper chaba* and demonstrated the presence of several important bioactive compounds. These findings support the traditional medicinal use of *Piper chaba* and agree with previous studies reporting that species of the genus *Piper* are rich sources of pharmacologically active phytochemicals (Parmar et al., 1997; Salehi et al., 2019).

The positive results obtained from Mayer's, Wagner's, Dragendorff's, and Hager's tests confirmed the presence of alkaloids in the methanolic extract. Alkaloids are one of the most important groups of secondary metabolites because they exhibit antimicrobial, analgesic, anti-inflammatory, antioxidant, antihypertensive, and anticancer properties (Harborne, 1998). The presence of alkaloids in *Piper chaba* may therefore contribute to its traditional use in treating respiratory infections, fever, gastrointestinal disorders, and inflammatory conditions. Previous phytochemical investigations of *Piper* species have also identified piperine and related alkaloids as major bioactive constituents responsible for many pharmacological activities (Srinivasan, 2007).

Flavonoids were detected by both alkaline reagent and lead acetate tests. Flavonoids are well-known natural antioxidants that protect cells against oxidative stress by scavenging reactive oxygen species. They also possess antimicrobial, antiviral, anti-inflammatory, antidiabetic, cardioprotective, and anticancer activities (Pandey & Rizvi, 2009). The presence of flavonoids in the methanolic extract suggests that *Piper chaba* may have significant antioxidant potential, which could partly explain its traditional medicinal applications.

The ferric chloride test confirmed the presence of phenolic compounds. Plant phenolics are among the most effective natural antioxidants because they inhibit lipid peroxidation, neutralize free radicals, and reduce oxidative damage associated with chronic diseases (Pandey & Rizvi, 2009). Numerous studies have demonstrated a positive relationship between total phenolic content and antioxidant activity in medicinal plants. Therefore, the presence of phenolic compounds in *Piper chaba* indicates its potential as a natural source of antioxidant agents.

Tannins were also detected in the methanolic extract through the gelatin test. Tannins are polyphenolic compounds recognized for their antimicrobial, astringent, antioxidant, and anti-inflammatory properties (Evans, 2009). These compounds inhibit the growth of microorganisms by interacting with microbial enzymes and cell membranes, thereby reducing microbial proliferation. The detection of tannins supports the traditional use of *Piper chaba* in the management of diarrhoea, dysentery, and wound infections.

The presence of glycosides and phytosterols further indicates the medicinal value of the plant. Glycosides have been reported to possess cardioprotective, antimicrobial, anti-inflammatory, and antioxidant activities, whereas phytosterols exhibit cholesterol-lowering, anti-inflammatory, and immunomodulatory effects (Evans, 2009). Positive results obtained from the Modified Borntrager's, Legal's, Salkowski's, and Liebermann–Burchard's tests confirm that these phytochemicals are important constituents of the methanolic extract.

Among the carbohydrate tests, only Benedict's test was positive, indicating the presence of reducing sugars. The negative Molisch's and Fehling's tests may be attributed to the relatively low concentration or specific nature of carbohydrates extracted by methanol. Similar variations in carbohydrate detection have been reported in qualitative phytochemical analyses due to differences in extraction solvents and plant composition (Harborne, 1998).

The absence of saponins, demonstrated by negative froth and foam tests, suggests that these compounds are either absent or present below the detectable concentration. Likewise, the negative ninhydrin reaction indicates that free amino acids were not present in measurable amounts. However, proteins were detected through the xanthoproteic test, suggesting that proteinaceous compounds are present even though free amino acids are lacking.

The findings of this study are generally consistent with previous reports describing the phytochemical composition of *Piper* species, including the presence of alkaloids, flavonoids, phenols, tannins, glycosides, and sterols (Parmar et al., 1997; Salehi et al., 2019). Minor differences between studies may be due to geographical location, climatic conditions, plant age, harvesting season, extraction solvent, and laboratory methodology, all of which can influence phytochemical composition (Azwanida, 2015).

Overall, the present investigation confirms that the methanolic extract of *Piper chaba* contains a diverse range of biologically active phytochemicals. These compounds are

likely responsible for the plant's traditional medicinal uses and suggest considerable potential for antioxidant, antimicrobial, anti-inflammatory, and other pharmacological activities. Further studies involving quantitative phytochemical estimation, chromatographic characterization, toxicity evaluation, and pharmacological investigations are recommended to identify the specific active constituents and validate their therapeutic efficacy.

5.0 CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

The present study was conducted to investigate the phytochemical constituents of the methanolic extract of the indigenous medicinal plant *Piper chaba* using standard qualitative phytochemical screening methods. The findings demonstrated that the methanolic extract contains a wide range of biologically active secondary metabolites, including alkaloids, glycosides, phytosterols, phenolic compounds, tannins, flavonoids, proteins, and reducing sugars. Conversely, saponins and free amino acids were not detected in the extract under the conditions employed in this investigation.

Among all the phytochemicals tested, alkaloids were strongly indicated by positive results in Mayer's, Wagner's, Dragendorff's, and Hager's tests, suggesting that they are among the principal constituents of the extract. Glycosides and phytosterols were also positively identified, indicating the potential presence of compounds with important pharmacological activities. The detection of flavonoids, phenolic compounds, and tannins suggests that *Piper chaba* possesses considerable antioxidant potential, as these phytochemicals are known to scavenge free radicals and protect against oxidative stress. The positive xanthoproteic test further confirmed the presence of proteins, while Benedict's test indicated the occurrence of reducing sugars.

The phytochemical profile obtained in this study supports the traditional medicinal use of *Piper chaba* in the treatment of various ailments, including inflammatory disorders, respiratory diseases, gastrointestinal problems, and microbial infections. The presence of these secondary metabolites provides a scientific basis for the plant's reported antimicrobial, antioxidant, anti-inflammatory, and other therapeutic properties. The study also demonstrates that methanol is an effective solvent for extracting a broad range of bioactive compounds from *Piper chaba*.

The methanolic extract of *Piper chaba* represents a valuable source of biologically active phytochemicals with potential pharmaceutical significance. The findings contribute to the scientific understanding of this indigenous medicinal plant and provide baseline information for future phytochemical and pharmacological investigations. However, qualitative screening alone cannot determine the concentration or specific chemical structures of the detected compounds. Therefore, further quantitative and advanced analytical studies are required to isolate, characterize, and evaluate the therapeutic potential of the active constituents.

5.2. Recommendations

Based on the findings of the present study, the following recommendations are proposed.

Quantitative phytochemical analysis should be conducted to determine the concentrations of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, and other important phytochemicals present in the methanolic extract of *Piper chaba*.

Pharmacological studies, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, analgesic, and anticancer assays, should be performed to validate the traditional medicinal uses of *Piper chaba*.

Toxicological and safety evaluations should be undertaken to determine the safety profile, therapeutic dosage, and possible adverse effects of the plant extract before considering clinical applications.

Future studies should focus on bioassay-guided isolation of active compounds responsible for the observed biological activities, facilitating the development of novel plant-based therapeutic agents.

Conservation and sustainable cultivation of *Piper chaba* should be encouraged to preserve this valuable indigenous medicinal plant and ensure its availability for future pharmaceutical and scientific research.

6. CONFLICTS OF INTERESTS

The authors do not disclose any conflicting interests.

7. ACKNOWLEDGMENT

The authors acknowledge Muhammad Yeasin Arafat for assistance with the experiments.

8. REFERENCE

1. Azwanida, N. N. (2015). *A review on the extraction methods used in medicinal plants, principle, strength and limitation*. *Medicinal & Aromatic Plants, 4*(3): 196.
2. Dyer, L. A., & Palmer, A. D. N. (Eds.). (2004). Piper: A model genus for studies of phytochemistry, ecology, and evolution. Springer.
3. Evans, W. C. (2009). *Trease and Evans' Pharmacognosy* (16th ed.). Saunders Elsevier.
4. Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd ed.). Chapman & Hall.
5. Islam, M. T., Hasan, J., Snigdha, H. M. S. H., Ali, E. S., & Sharifi-Rad, J. (2020). Chemical profile, traditional uses, and biological activities of Piper chaba Hunter: A review. Journal of Ethnopharmacology, 257: 112853. https://doi.org/10.1016/j.jep.2020.112853
6. Kew Science. (2026). Plants of the World Online: Chavica chaba Miq. (synonym of Piper retrofractum). https://powo.science.kew.org/
7. Khare, C. P. (2007). *Indian medicinal plants: An illustrated dictionary*. Springer.
8. National Center for Biotechnology Information. (2023). Piper retrofractum (syn. Piper chaba): Taxonomy and botanical information. https://www.ncbi.nlm.nih.gov/
9. Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over nearly four decades (1981–2019). Journal of Natural Products, 83(3): 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285
10. Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity, 2*(5): 270–278.
11. Parmar, V. S., Jain, S. C., Bisht, K. S., Jain, R., Taneja, P., Jha, A., Tyagi, O. D., Prasad, A. K., Wengel, J., Olsen, C. E., & Boll, P. M. (1997). Phytochemistry of the genus Piper. Phytochemistry, 46(4): 597–673.
12. Plants of the World Online. (2024). Piper retrofractum Vahl. Royal Botanic Gardens, Kew. https://powo.science.kew.org/
13. Rahman, A. H. M. M. (2013). Traditional medicinal plants used by local people of Bangladesh. *International Journal of Biodiversity and Conservation, 5*(11): 685–692.
14. Rahman, M., Islam, M. T., & colleagues. (2024). Comparison of piperine content, antimicrobial and antioxidant activity of Piper chaba root and stem. BMC Complementary Medicine and Therapies. https://pmc.ncbi.nlm.nih.gov/articles/PMC11483291/

15. Rameshkumar, K. B., Aravind, A. P. A., & Mathew, P. J. (2011). Comparative phytochemical evaluation and antioxidant assay of Piper longum L. and Piper chaba Hunter used in Indian traditional systems of medicine. Journal of Herbs, Spices & Medicinal Plants, 17(4): 351–360.
16. Salehi, B., Zakaria, Z. A., Gyawali, R., Ibrahim, S. A., Rajkovic, J., Shinwari, Z. K., Khan, T., Sharifi-Rad, J., Ozleyen, A., Turkdonmez, E., Valussi, M., Tumer, T. B., Monzote, L., Martorell, M., & Setzer, W. N. (2019). Piper species: A comprehensive review on their phytochemistry, biological activities and applications. Molecules, 24(7): 1364. https://doi.org/10.3390/molecules24071364
17. Salehi, B., Zakaria, Z. A., Gyawali, R., Ibrahim, S. A., Rajkovic, J., Shinwari, Z. K., Khan, T., Sharifi-Rad, J., Ozleyen, A., Turkdonmez, E., Valussi, M., & Martorell, M. (2019). Piper species: A comprehensive review on their phytochemistry, biological activities, and applications. Molecules, 24(7): 1364. https://doi.org/10.3390/molecules24071364
18. Sofowora, A. (2008). *Medicinal plants and traditional medicine in Africa* (3rd ed.). Spectrum Books Limited.
19. Srinivasan, K. (2007). Black pepper and its pungent principle piperine: A review of diverse physiological effects. Critical Reviews in Food Science and Nutrition, 47(8): 735–748. https://doi.org/10.1080/10408390601062054
20. Trease, G. E., & Evans, W. C. (2009). *Trease and Evans' pharmacognosy* (16th ed.). Saunders Elsevier.
21. World Health Organization. (2013). *WHO traditional medicine strategy: 2014–2023*. World Health Organization.