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ORIGINAL ARTICLE

Qualitative Estimation of Piperine from Various Parts of the *Piper chaba* Hunt.

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ABSTRACT
There has been an increasing interest in discovering biologically active natural compounds derived from medicinal plants because they are perceived to be safer and more effective than synthetic chemicals. Piper chaba Hunt. a member of the Piperaceae family is a relatively lesser-known plant that is used both as a spice and for medicinal purposes. It has shown potential efficacy in treating colds and coughs. The objective of this study is to determine the qualitative analysis of piperine the main bioactive component of this plant that is responsible for its therapeutic characteristics. Various parts of the P. chaba plant, such as the root, stem, leaves, and spike were subjected to phytochemical analysis using five different solvents (methanol, ethanol, acetone, chloroform, and ethyl acetate) revealing the presence of alkaloids in these plant parts. A thin-layer chromatography (TLC) analysis was used to assess piperine in these extracts qualitatively. All solvents showed consistent piperine levels in the spike and stem, but variations were seen in the roots and leaves. The R_f values ranged from 0.51 to 0.57, which illustrates slight differences in the solubility of piperine across solvents. The results of this study show the importance of optimizing the extraction methods for bioactive compounds in P. chaba, contributing to the standardization of the alkaloid content of this plant and its potential therapeutic application.
Keywords*: Piperine, TLC, Bioactive compound, Piper chaba, R_f value.*

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INTRODUCTION

Over the past few decades, plant-derived active ingredients and compounds have gained significant attention due to their extensive chemical diversity. The demand for herbal medicines has increased as a result of this growing interest, underscoring the necessity of assuring their efficacy, safety, and quality [1]. The manufacture of pharmaceuticals and medicines for the benefit of humans and other living things frequently uses medicinal plants, which are rich sources of diverse bioactive chemicals [2]. An important aspect of quality evaluation is phytochemical assessment, which includes preliminary screening, chromatographic fingerprinting, and marker compound analysis. Several techniques can be used to analyze these components, including high-performance liquid chromatography (HPLC), gas chromatography (GC), and thin-layer chromatography (TLC). Specifically, TLC is a traditional, easy, effective, and inexpensive method that is frequently used to separate various constituents in a mixture [3]. It is extensively utilized for the qualitative identification of trace contaminants and the investigation of natural medications in pharmacopeias.

An essential alkaloid found in many pepper species, piperine has several noteworthy pharmacological properties, such as antidepressant and cognitive-improving properties [4, 5]. *Piper chaba* Hunt., identified as a major source of piperine, is a flowering vine from the Piperaceae family, native to South and Southeast Asia. It is commonly known as Choi Jhal in Bangladesh and Badi Pippali in India [6]. *P. chaba* is frequently utilized in traditional medicine as an ethnopharmacological treatment for a variety of illnesses. The fruit of *P. chaba* has stimulating and carminative qualities that help relieve hemorrhoidal symptoms, while the root is recognized for its alexiteric properties, which make it effective in treating bronchitis, asthma, and consumption. The stem is used to relieve diarrhea, rheumatic pain, and postpartum

discomfort [7]. There are a variety of antibacterial, carminative, expectorant, analgesic, hypotensive, and relaxing effects of crude extracts of *P. chaba*, highlighting the plant's high content of amides and alkaloids [8]. Alkaloids include piperine, piperanine, pipernonaline, piperamine 2, 4-decadienoic acid piperidide, kusunokinin, and pellitorine are among the significant bioactive components found in *P. chaba* [9].

Several of these compounds have shown promise as antimicrobial agents, inhibited Gram-positive and Gram-negative bacteria growth, as well as possessed strong insecticidal properties. These compounds represent a natural alternative to synthetic insecticides. It is crucial to find approachable techniques for analyzing these chemicals to discover new drugs, given the medicinal potential of *P. chaba* and the increasing interest in its bioactive components. Because of the complexity of the molecules involved, bioactive substances from plant extracts can be difficult to analyze, characterize, and structurally elucidate [10]. For the successful extraction and isolation of these bioactive chemicals, analytical technique optimization is essential. It has been emphasized by the World Health Organization (WHO) that modern, controlled techniques must be used to ensure the quality of medicinal plant products [11]. For the standardization of natural product drugs, single chemical entities, or "marker compounds," can be used as potency standards in TLC analysis. This study aims to develop a TLC method for the qualitative analysis of piperine in *P. chaba* root, stem, leaves, and spike extracts, focusing on optimizing the separation of the primary components in these extracts.

MATERIAL AND METHODS

Procurement, Collection, and processing of plant materials

In January 2023, samples from a fully matured, 2-year-old *P. chaba* vine, including the root, stem, leaves, and spike, were collected from the Herbal Garden at Dayalbagh Educational Institute (DEI) in Dayalbagh, Agra. The freshly harvested plant samples were carefully rinsed first with groundwater and then with sterilized distilled water to remove any adhering dirt. The samples were then air-dried in a shaded area at room temperature (25°C) for 12 days to preserve the active compounds. After drying, the samples were crushed into small pieces and pulverized with a machine grinder. The ground samples were sieved through a 1 mm pore size sieve and prepared for reflux extraction by placing them in a thimble.

Plant extracts preparation for analysis

The plant samples were extracted using a refluxing method. Each plant part including root, stem, leaves, and spike was separately extracted with five different solvents: ethanol, methanol, acetone, chloroform, and ethyl acetate. For each extraction, 10 grams of the powdered plant material was placed in a thimble and refluxed with 100 mL of the chosen solvent for about 6 hours at 40°C. After refluxing, the crude extracts were filtered through Whatman No.1 filter paper, concentrated under reduced pressure and low temperatures, and then stored at 4°C for further analysis.

Test for alkaloids

Mayer's test: 1 ml of the extract was mixed with 2 ml of Mayer's reagent. The appearance of a pale whitish precipitate indicated the presence of alkaloids [12].

Qualitative test for alkaloid screening of various parts of *P. chaba*

Thin Layer Chromatography

The thin-layer chromatography method (TLC) is an analytical technique for separating molecules based on their interaction with a stationary phase and a mobile phase. The mobile phase is a liquid solvent that passes through the stationary phase, which is a thin layer of silica gel spread out on a glass plate that serves as an adsorbent. As solutes move through the stationary phase, this procedure makes it possible to identify and separate them. Kolhe et al. [13] used TLC to validate a chemical alkaloid test in various extracts to confirm the presence of piperine.

Mobile phase preparation

A chromatographic method was performed using toluene (Merck) and ethyl acetate (Merck) as mobile phases. An optimal separation of alkaloids was achieved using a 70:30 ethyl acetate toluene ratio.

Preparation of standard solution

Piperine (Sigma-Aldrich) is dissolved in 1 ml of methanol to obtain the standard solution with a concentration of 1 mg/ml.

Preparation of sample solution

The plant extracts were reduced by rotary evaporation under low temperatures and reduced pressure after extraction. Plant extracts were then selected in a precise quantity for subsequent chromatography.

Plate for thin-layer chromatography

Chromatography was performed using TLC silica gel 60 F_{260} on an aluminum sheet measuring 7.5 cm by 10 cm (Merck, Germany).

Thin layer chromatography method for piperine detection

All five extracts of *P. chaba* were subjected to TLC analysis. 2 µl of all extracts were manually spotted onto TLC plates for each study, with the spots being set 1.5 cm from the plate's side and 1 cm from the bottom. There were four points on each plate, each 1.5 cm apart, and the application parameters remained the same for every analysis. All of the TLC plates utilized in the investigation were identical. After that, the spotted plates were put within a glass chamber that had been previously saturated with a toluene and ethyl acetate mobile phase solvent mixture. The plates were partially immersed in the solvent until the solvent front reached saturation. Afterward, the plates were removed from the chamber and allowed to dry.

The dried plates were examined under ultraviolet (UV) light to detect piperine. Iodine vapor post-development derivatization allowed for additional identification of the emerging areas. To ensure the accuracy of the results, a piperine standard was run concurrently with the test samples. Notable indications of piperine content were noted in distinct regions of the different samples (Figure 1). Mehta et al. [14] outlined the methodology used to determine and analyze the R_f values, which stand for the migration distances of the compounds on the TLC plates.

RESULTS

The phytochemical screening of different parts of the *P. chaba* plant was done to identify many bioactive compounds. Positive results were found in every part of the plant when Mayer's test was used to identify alkaloids in different *P. chaba* extracts. When evaluated with methanol, ethanol, acetone, chloroform, and ethyl acetate extracts, alkaloids were found in the extracts of the root, stem, leaves, and spike (Table 1).

Table 1: Results of the Mayer's test in various part of *Piper chaba* Hunt.

Phytochemical	Test	Methanol	Ethanol	Acetone	Chloroform	Ethyl Acetate
Alkaloids	Root	++	+	++	+	+
	Stem	++	++	+	+	++
	Leaves	++	++	++	+	+
	Spike	++	++	++	++	++

Piperine was found in the leaves extract of methanol, acetone, and chloroform, but not in the extracts of ethanol or ethyl acetate. All extracts of the stem revealed the presence of piperine. Piperine was found in the root's methanol and ethyl acetate extracts, but not in the extracts made of ethanol, chloroform, or acetone. The spike showed that piperine was present in every extract across the solvents used (Figure 1). Different solvents showed consistent R_f values, ranging from 0.51 to 0.57 (Table 2). Specifically, the R_f values of methanol extract showed an R_f value of 0.57, ethanol had an R_f value of 0.56, acetone showed 0.51, and chloroform and ethyl acetate had 0.53 and 0.52 respectively. These findings suggest that piperine exhibits varying degrees of solubility in different solvents across different parts of the plant, with the highest consistency in the methanol and ethanol extracts.

Table 2: Analysis of Piperine in different plant parts and extracts by TLC.

Extract / Plant part	Methanol	Ethanol	Acetone	Chloroform	Ethyl Acetate
Leaves	+	-	+	+	-
Stem	+	+	+	+	+
Root	+	-	-	-	+
Spike	+	+	+	+	+
R_f value	0.57	0.56	0.51	0.53	0.52

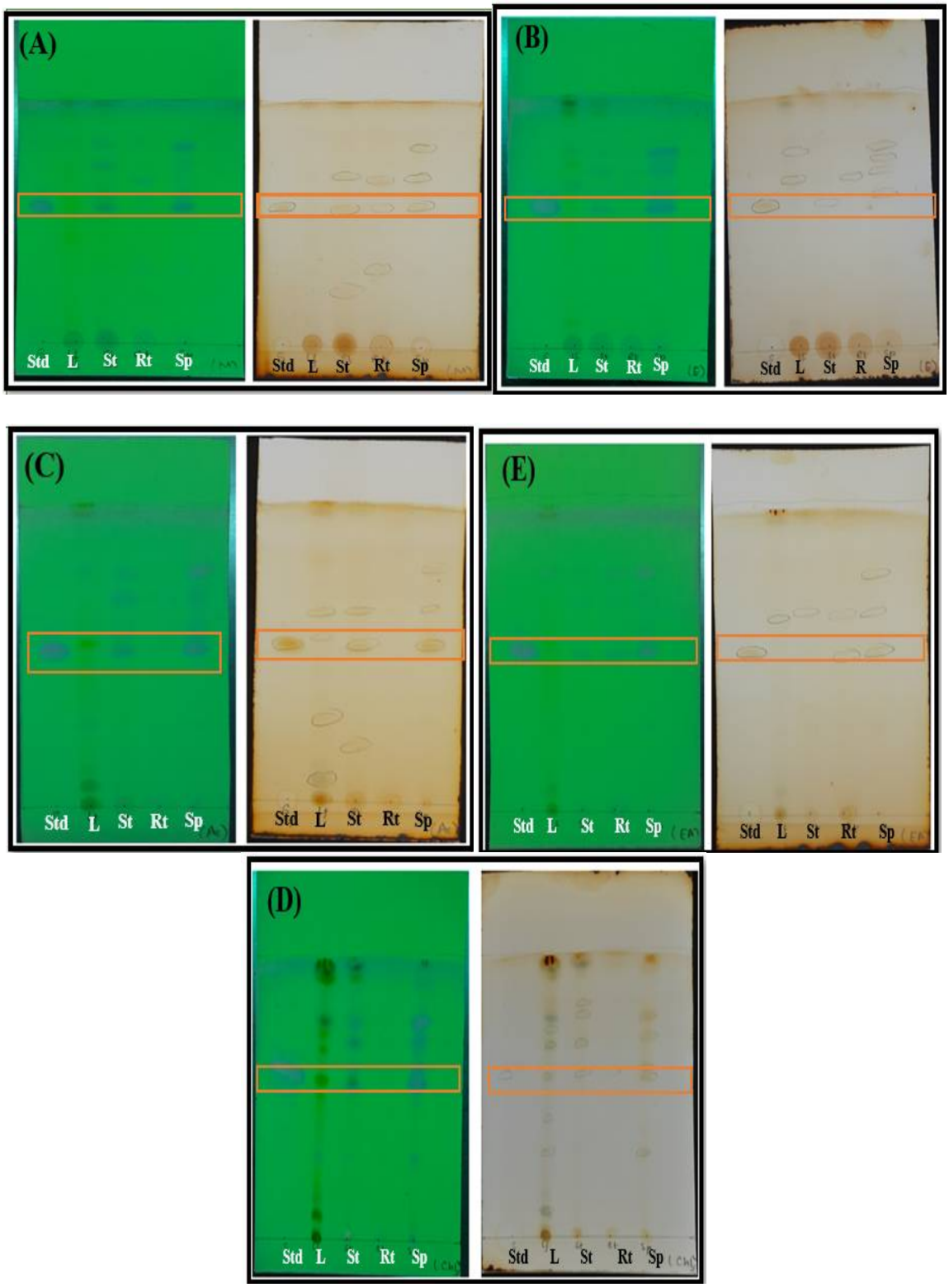

Figure 1: Separation of the compound by using thin layer chromatography of Leaves, stem, root, and spike of *Piper chaba* in different plant extract. (A) Methanolic Extract (B) Ethanolic Extract (C) Acetone Extract (D) Ethyl Acetate Extract (E) Chloroform Extract. [Std- Standard, L- Leaves, St- Stem, Rt- Root, Sp- Spike]

DISCUSSION

The presence of various bioactive compounds, including alkaloids, which are frequently present across different species within the genus, has been discovered by phytochemical evaluation of different parts of

the *P. chaba* plant. Well-known alkaloid piperine has been found in significant amounts in the roots and spikes of allied species like *P. nigrum, P. longum, and P. cubeba* [15-19]. Similarly, comprehensive phytochemical profiling has been conducted on *Psidium guajava, Allium sativum*, *Tribulus terrestris*, *Pedalium murex,* and *Curcuma longa* for analyzing various bioactive components [20-22]. In *Pedalium murex*, the distribution of the alkaloids, phenolics, saponins, and flavonoids has been noted in the ethanol extract [23]. The distribution of piperine in *P. chaba* has been the primary objective of the current study. To find out whether alkaloids were present in different plant parts, standard phytochemical assays were used [24, 25]. Meyer's test results revealed that alkaloids were present in each part of *P. chaba* that was analyzed. Because herbal remedies are naturally complex, it might be difficult to identify every component of them. A popular method for learning more about the polarity and distribution of chemical components in plant extracts is thin-layer chromatography (TLC). According to the study by Kumar et al. [26], compounds with higher R_f values in less polar solvent systems are typically less polar, whereas those with lower R_f values are more polar. The TLC analysis of *P. chaba* revealed varying levels of piperine presence across different plant parts and solvent systems, a finding that parallels observations in other *Piper* species. The present investigation revealed the presence of piperine in methanol, acetone, and chloroform extracts of the leaves. The R_f values of 0.57 and 0.56 for methanol and ethanol extracts, respectively, indicated comparable piperine content. It is compatible with the findings in *P. longum*, where piperine was extracted in a similar manner utilizing a variety of solvents, including methanol and ethanol [27]. This pattern of piperine detection in numerous solvents is consistent with the results obtained.

Piperine is widely distributed in all solvent extracts of *P. chaba* stems, similar to the results found in *P. nigrum*, where piperine was evenly dispersed throughout all solvent extracts [28]. The presence of piperine was detected in methanol and ethyl acetate extracts of the roots, but not in ethanol, chloroform, or acetone extracts. There are differences in R_f values for ethyl acetate (0.52) and methanol (0.57) based on changes in the chemical constituents of root extracts, which have previously been reported in *P. betle*. According to Jayalakshmi et al [29], some alkaloids can be selectively extracted depending on the solvent used. Piperine was consistently found in all solvent extracts of the spike, indicating a higher piperine concentration or easier extraction from it. The constant R_f values across solvents, similar to the stem, imply a stable polarity of piperine within the spike. The observed differences in extraction efficiency among plant sections and solvents indicate that particular solvents are more effective in isolating piperine from various plant tissues. Methanol was effective in extracting piperine from the leaves and root, while the stem and spike were more extractable across all solvents tested. Several *Piper* species exhibit similar variations in piperine distribution, suggesting distinctive metabolic functions and chemical compositions [30, 31]. The consistency of piperine in the spike and stem of the plant suggests that they can be used for piperine extraction regardless of the solvent used.

CONCLUSION

This study successfully detected piperine, a significant bioactive alkaloid found in *P. chaba* parts, using thin-layer chromatography (TLC) and several solvent extraction techniques. Plant phytochemical screening and TLC analysis confirmed the presence of piperine and revealed that different solvents extract this component differently from various plant parts. It was found that methanol performed effectively as a solvent to extract piperine from leaves and roots. The comparatively uniform Rf values among the several solvents indicate that the polarity of the solvent does not affect piperine migration in TLC. The present study has shown that *P. chaba* has the potential as a valuable source of piperine which can be used in the development of nutraceuticals and pharmaceutical formulations.

AUTHOR CONTRIBUTIONS

DS designed the study and wrote the manuscript. The manuscript was edited by RR. Both authors have reviewed and approved this manuscript.

CONFLICT OF INTEREST

There is no conflict of interest declared by the authors.

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