



Preliminary Phytochemical, Physicochemical and Quantitative Evaluation of *Piper nigrum* Fruit

Bhalerao Sachin Shrihari, Sanket Dharashivkar and Rajeev Malviya*

School of Pharmacy, Mansarovar Global University, Kolar Road, Bhopal (M.P.), India

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Abstract

This study focuses on the preliminary phytochemical, physicochemical, and quantitative evaluation of *Piper nigrum* fruit. Various extraction and analysis techniques were employed to determine the plant's chemical composition, physicochemical parameters, and active constituent content. Results revealed the presence of key phytochemicals like alkaloids, flavonoids, tannins, and volatile oils. Physicochemical parameters, including moisture content, ash values, and extractive values, were found within pharmacopoeial limits. The active constituent piperine was quantified using HPLC and TLC methods, demonstrating the efficacy of both laboratory-prepared and marketed extracts. The study provides a foundational understanding of the bioactive compounds in *Piper nigrum* and its potential therapeutic applications.

Keywords: *Piper nigrum*, phytochemical screening, physicochemical evaluation, piperine, HPLC, TLC

Introduction

Medicinal plants have served as a cornerstone of traditional medicine and modern pharmacology, offering a vast array of bioactive compounds that hold therapeutic potential. Among these plants, *Piper nigrum* (commonly known as black pepper) occupies a significant position due to its culinary and medicinal value. Known as the "King of Spices," *Piper nigrum* is extensively cultivated in tropical regions and has been a vital component of traditional medicine systems like Ayurveda, Unani, and Traditional Chinese Medicine [1][2]. The fruit of *Piper nigrum* is a rich source of bioactive compounds, with piperine being the primary alkaloid responsible for its pungency and pharmacological activities. Piperine exhibits a wide range of therapeutic properties, including antioxidant, anti-inflammatory, antimicrobial, and bioavailability-enhancing effects [3][4].

Additionally, the presence of flavonoids, tannins, terpenoids, and volatile oils further enhances the medicinal profile of *Piper nigrum* [5][6].

The growing global demand for herbal remedies underscores the need for systematic evaluation of medicinal plants to ensure quality, safety, and efficacy. Preliminary phytochemical screening helps identify the active constituents present in plant materials, while physicochemical and quantitative analyses ensure the consistency and authenticity of the herbal product [7][8]. For *Piper nigrum*, such studies provide insights into its chemical profile and establish a scientific basis for its traditional uses.

***Corresponding Author**

E-mail: rajeevr33@gmail.com

Despite its widespread use, there remains a need for comprehensive studies that integrate phytochemical, physicochemical, and quantitative evaluations of *Piper nigrum*. This study addresses this gap by employing validated methods to characterize the chemical composition and physicochemical properties of *Piper nigrum* fruit. The findings aim to support its standardization and therapeutic applications while providing a foundation for further pharmacological and clinical studies [9][10].

Through this research, we aim to reinforce the scientific understanding of *Piper nigrum*'s medicinal potential and contribute to its broader acceptance in both traditional and modern healthcare systems.

This study aimed to investigate the phytochemical profile, physicochemical properties, and quantitative composition of *Piper nigrum* fruit extracts.

Material and Method

Collection and Authentication of Plant Material

The fruits of *Piper nigrum* were collected from a reputable source and authenticated by a certified botanist. A voucher specimen was retained in the department for future reference.

Preparation of Extract

The extraction process followed the methodology described by Yang Zo et al. [5] with slight modifications. The fruits were washed, shade-dried, powdered, and defatted using petroleum ether. The defatted material was extracted with ethanol using a Soxhlet apparatus, and the ethanol was evaporated to obtain a dried extract.

Preliminary Phytochemical Screening

The prepared extract underwent qualitative tests for alkaloids, flavonoids, tannins, terpenoids, saponins, phenols, and other phytochemical constituents using standard methods [6][7].

Physicochemical Evaluation

Physicochemical parameters, including loss on drying (LOD), total ash, acid-insoluble ash, and extractive values (water and alcohol soluble), were determined as per the Ayurvedic Pharmacopoeia of India guidelines [8].

Thin Layer Chromatography (TLC)

TLC was performed using toluene:ethyl acetate (7:3) as the solvent system and vanillin-sulfuric acid reagent for derivatization. The R_f value of

piperine was identified under UV light at 254 nm and 366 nm [9].

High-Performance Liquid Chromatography (HPLC)

Quantitative estimation of piperine content was conducted using HPLC. The percentage composition was determined for both laboratory-prepared and marketed extracts [10].

Results and Discussion

Phytochemical Screening

Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, and volatile oils in *Piper nigrum* extract (Table 1).

Table 1: Phytochemical Constituents of *Piper nigrum*

Constituent	Presence (+)
Alkaloids	+
Flavonoids	+
Tannins	+
Terpenoids	+
Saponins	+
Volatile Oils	+

Physicochemical Evaluation

Physicochemical parameters of *Piper nigrum* are summarized in Table 2. All values complied with pharmacopoeial standards, indicating proper storage and minimal contamination.

Table 2: Physicochemical Parameters of *Piper nigrum*

Parameter	Value	Standard Limit
Moisture Content (LOD)	$3.18 \pm 0.27\%$	NMT 5%
Total Ash	$4.59 \pm 0.19\%$	NMT 5%
Acid-Insoluble Ash	$0.70 \pm 0.17\%$	NMT 1%
Water-Soluble Extractive	$13.33 \pm 1.50\%$	NLT 6%
Alcohol-Soluble Extractive	$9.00 \pm 2.11\%$	NLT 6%

Quantitative Analysis of Piperine

TLC and HPLC analysis revealed consistent piperine content between laboratory-prepared and marketed extracts. Thin layer chromatography was the basic technique for the herbal drug to determination of active constituent. From the TLC, R_f values were found 0.29 piperine

respectively in lab and market extracts (Table 3). to marker compound as per Wagner et al⁴.
R_f values of all herbal extracts were found similar

Table 3: TLC fingerprinting data

Drug	Mobile Phase/Solvent System	Derivatizing reagent	Visualizations	No. of Spots	R _f Values of Bands
<i>Piper nigrum</i>	Toluene: Ethylacetate(7:3)	Vanillin- H ₂ SO ₄ reagent	Under 254 nm	1	0.29
			Under 366 nm	1	
			Under 366 nm	1	

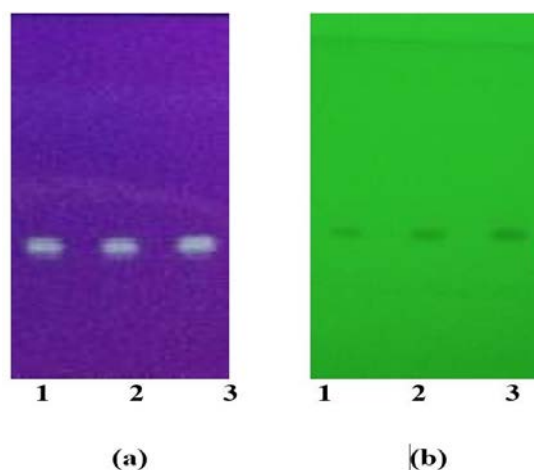


Figure 1: TLC of *Piper nigrum* (a) Under 366 nm (b) Under 254 nm
(1) Standard piperine (2) Lab methanolic extract (3) Market methanolic extract

From the UV and HPLC study, %w/w of active constitutes was determined in market and lab extract of herbal drugs (Table no 4). It was found that higher percentage of active constitutes shown in market extract of all herbal drugs. Hence,

market extract was used in further studies. Also, their respective HPLC chromatograms of standard, marketed and lab extract were shown in figure 5.8, 5.9 and 5.10.

Table 4: % w/w of active constituents in herbal drugs

Active constituent Piperine	Method	<i>Piper nigrum</i>	
		Lab	Market
	HPLC	26.52%	28%
		38.5%	50.27%

The presence of diverse bioactive compounds supports the medicinal significance of *Piper*

nigrum. The high piperine content, verified through HPLC, underscores its potential for pharmaceutical applications [11][12].

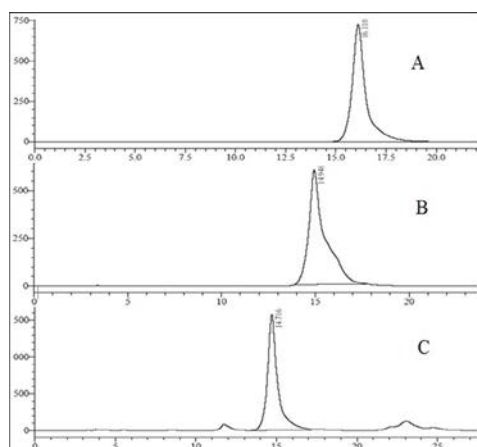


Figure2: HPLC chromatogram of (A) Standard piperine (B) Market extract (C) Lab extract of *Piper nigrum*

Conclusion

The preliminary phytochemical, physicochemical, and quantitative evaluation of *Piper nigrum* confirmed the presence of key bioactive compounds, notably piperine. Physicochemical parameters were within pharmacopoeial limits, and quantitative analysis demonstrated the extract's consistency and quality. This study establishes a foundation for further research into the therapeutic applications of *Piper nigrum*.

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