HPTLC Fingerprinting and Antimicrobial Analysis of Trikatu: A Comprehensive In-Vitro Study

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Abstract- Piper nigrum and Piper longum both belonging to family Piperaceae and Zingiber officinale belonging to Zingiberaceae family, are well known in traditional medicine. Trikatu is a traditional herbal formulation containing three herbs mixed together in equal quantities. Trikatu churma plays an important role in balancing respiratory and digestive health. The major aim of present study was to study the antimicrobial activity and chromatographic evaluation of Trikatu to scientifically validate its traditional uses.

The chromatographic evaluation of Trikatu using High-Performance Thin-Layer Chromatography (HPTLC) to identify key phytochemical markers. Distinct bands corresponding to major bioactive compounds were observed, enabling comparative profiling and confirmation of formulation integrity. The developed HPTLC fingerprint provides a reliable tool for routine quality assessment, authentication, and standardization of Trikatu.

Extract of Trikatu i.e. P. nigrum, P. longum and Z. officinale were tested for their antimicrobial activity by agar well diffusion method. The Methanol, Hexane and Aqueous extract of the

Trikatu was studied against Staphylococcus aureus and Escherichia coli as test microorganisms. The results reveal that the Methanol has shown significant activity against Staphylococcus aureus and Escherichia coli as compared to Hexane and Aqueous extract indicating the plant can restrain these organisms.

This project also explores the antiviral relevance of Trikatu by reviewing ancient textual references and correlating them with modern antiviral pharmacology. References such as the Vedas, Puranas, and Ayurvedic Samhitas. Through a comparative analysis of scriptural descriptions and current scientific literature, this study proposes that the synergistic phytoconstituents of Trikatu may exhibit antiviral actions through immunomodulation, bio enhancement, and respiratory health support.

Key words: Piper nigrum, Piper longum, Zingiber officinale, Antimicrobial, Chromatographic evaluation, Well diffusion, Staphylococcus aureus, Escherichia coli, Synergistic phytoconstituents, Antiviral actions, Immunomodulation, Bio enhancement.

Introduction

The word Trikatu is derived from a Sanskrit word where "Tri" means three and "Katu" means bitter. Trikatu is a traditional herbal formulation containing three herbs mixed together in equal quantities. These three herbs are Marich (Piper nigrum), Pippali (Piper longum), and Sunthi (Zingiber officinale). Trikatu churma plays an important role in balancing respiratory and digestive health. In stomach, it increases production of digestive juices thereby stimulating digestion. It has a powerful effect on mucous membrane of the gastrointestinal tract and regulates the intestinal functions to facilitate absorption. Trikatu acts primarily by its effect on stomach, liver, and pancreas. In liver, it acts as Cholagogue and increases production of bile salts by stimulating gallbladder functioning.

Dosage: Indigestion & Bloating ¼ to ½ teaspoon of Trikatu powder with warm water before meals. Weight Loss ½ teaspoon Trikatu with honey in the morning. Cough & Cold Mix ¼ teaspoon Trikatu with honey and consume twice daily. Joint Pain & Inflammation ¼ teaspoon Trikatu powder with warm turmeric milk before bedtime.

Precaution: Trikatu may worsen gastritis and cause a burning sensation if taken in higher doses due to its hot potency. It is not recommended for pregnant and breastfeeding women without a doctor's consultation.

Piper nigrum

P. nigrum belonging to the family Piperaceae is commonly known as "Maricha", is a climber plant. Maricha is formed by drying the green unripe fruit of the pepper plant. For many days it is Sun-dried or machine-dried, during which pepper gains black wrinkled skin. Maricha is an herb which can aggravate pitta. Black Pepper is exceedingly a very popular and important ingredient that is used in cooking worldwide. It is usually used in flavouring food but beside this the herb provides medicinal benefits. It has ability to improve the function of the digestive system, which reduces flatulence and bloating along with other medicinal properties.

Piper longum

Long pepper or Pippali is a fragrant, thin vine with creeping, segmented stems. Its fleshy fruits are set into elongated spikes. Spike turns red in colour once ripened. Long pepper has its reference from the ancient textbooks of Ayurveda. Ayurveda has explained various uses of long pepper for dietary purposes as well as for various health purposes. It is basically used for a healthy respiratory system. Long pepper is also beneficial for healthy digestion and healthy metabolism as well. Pippali is a useful herb for cold and

mucus conditions of the lungs as it boosts vasodilatation and increases circulation to the lung. It acts as a bronchodilator, decongestant and expectorant. Pippali is a strong rejuvenative for the lungs. This herb is recommended as it will prevent colds.

Zingiber officinale

Zingiber officinale is a flowering plant, which is commonly known as ginger. It belongs to the Zingiberaceae family. The Indian name of this plant is dry ginger or Saunth/ Sunthi or Adrak. It is very commonly used spice. Gingerols is its natural oil that provides a unique flavour and aroma to this spice. Ginger is famous in medicine system because of its medicinal properties. It has very powerful anti-inflammatory and anti-oxidant properties.

Chemical Composition

Piper nigrum and Piper longum belonging to the family Piperaceae has a distinct sharp flavour attributed to the presence of the phytochemical, piperine. Piperine, the active compound in Black pepper and long pepper offers several health benefits, including enhancing nutrient absorption, reducing inflammation and acting as an antioxidant. Piperine displays numerous pharmacological effects such as antioxidant, antimicrobial, and immunomodulatory effects in various in vitro and in vivo experimental trials.

Gingerols and Shogaols are the major pungent compounds present in the rhizomes of ginger i.e. Zingiber officinale and are renowned for their contribution to human health and nutrition. They have anti-inflammatory and digestive stimulant properties. Both gingerols and shogaols exhibit a host of biological activities, ranging from anticancer, anti-oxidant, antimicrobial, anti-inflammatory and anti- allergic to various central nervous system activities.

In Ayurvedic practice, Trikatu is primarily used to stimulate "Agni" i.e. the digestive fire and eliminate "Ama" i.e. undigested toxins.

Present work was carried out with the objective of Chromatographic evaluation and investigate antimicrobial activity of different solvent extracts of Trikatu against S. aureus and E. coli.

Material and Methods

Samples and Chemicals- Herbal sample of Trikatu, Solvents: Methanol, Ethanol, Chloroform, Distilled water

Phases- a. Stationary phase: Pre-coated silica gel 60 F254 plates (10×10 cm)
b. Mobile phase: Chloroform: Methanol (90:10)

Instruments- a. HPTLC applicator (Linomat)Twin-trough developing chamber
b. UV cabinet (254/366 nm)
c. CAMAG TLC Visualiser 3
d. Computer (VisionCATS Software)
e. Analytical balance
f. Water bath

Accessories- a. Microliter syringe
b. Whatman filter paper
c. Glassware: Volumetric flasks, Conical flasks, round bottom flasks, Measuring cylinder.

Chromatographic evaluation of Trikatu

High-Performance thin layer chromatography serves as an extension of thin-layer chromatography, offering robustness, simplicity, speed, and efficiency in the qualitative analysis of compounds.

The three herbs of Trikatu were collected and Grinded into fine powder. Each herb powder was mixed in equal proportion. The sample extract was prepared by accurately weighing 20 g of Trikatu and dissolving it in 150 mL Methanol, Ethanol and Aqueous solvent respectively. The solution was mixed well. After mixing, the solution was filtered through Whatman filter paper to obtain a clear sample extract suitable for chromatographic analysis. To concentrate the sample, it was put in the water bath.

For chromatographic separation, pre-coated silica gel 60 F254 plates were selected as the stationary phase. The sample extract was then applied on the plates using a CAMAG Linomat applicator. Application was done in the form of measured bands of 12 mm in width, ensuring uniform distance from the edges of the plate and uniform spacing between them.

A suitable mobile phase was selected. The mobile phase components i.e. Chloroform and Methanol (90:10) were mixed and poured into a twin-trough glass developing chamber. To ensure a stable and uniform saturation, the chamber was lined with saturation pads. Then the prepared Silica gel plate was carefully placed inside the chamber, ensuring that the sample application line remained above the mobile phase level. Development was allowed to occur until the solvent reached a marked distance of 8.5 cm. The plate was then removed and dried.

Once dried, the developed plate was observed under TLC visualizer. UV light was used at 254 nm and 366 nm to visualize the bands. The Vision CATS software generated TLC bands. These data were used for qualitative analysis.

Observation

The fingerprint profile generated from the HPTLC analysis provided information on the chemical composition, purity, and consistency of the herbal sample. The clear bands indicate good quality of raw material and absence of adulteration.

Antimicrobial activity of Trikatu

The agar well diffusion method is used to evaluate the antimicrobial activity of a Trikatu on test microorganisms i.e. S. aureus (gram positive) and E. coli (gram negative) bacteria. Two separate Conical flasks of 250 mL were taken. Agar medium was prepared measuring 6g Nutrient agar medium (Mueller Hinton agar) and dissolved it 200 mL Purified

water. The medium was then sterilized in an autoclave at 121°C for 30 minutes. After cooling the medium, grown bacteria of S. aureus and E. coli from separate test tube was inoculated using an inoculating needle. Bacteria inoculated was then suspended into the Nutrient agar medium and labelled.

The nutrient agar medium was then poured in 8 petri dishes (4 for S. aureus and 4 for E. coli) and allowed to solidify. Wells of 6–8 mm diameter were made aseptically in the agar using a sterile cork borer. Each well was filled with Trikatu extract of Methanol, Hexane and Aqueous solvent. 2 petri dishes were filled with positive control i.e. Methanol. After loading the wells, the plates were left undisturbed diffusion of the extract into the agar.

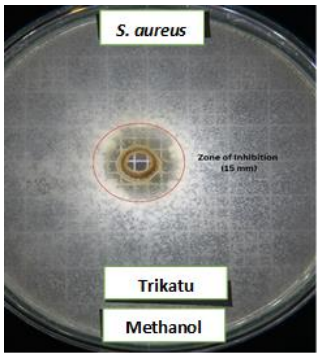
The prepared plates were incubated at 37°C for 18–24 hours. After incubation, the plates were examined for the presence of clear zones around the wells, indicating inhibition of bacterial growth. The diameter of each zone of inhibition was measured in millimetres using a transparent ruler. Larger inhibition zones indicated stronger anti-microbial activity. The results were recorded and compared across samples and controls, and concentrations to assess the potency of Trikatu against both bacterial strains.

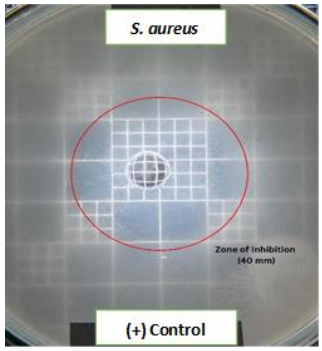
Table-1 ***Staphylococcus aureus*** (Gram positive bacteria)

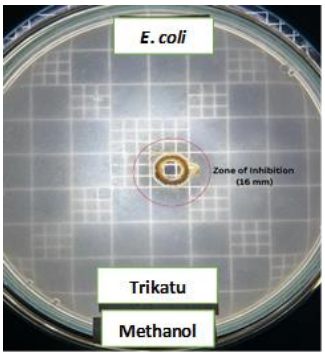
S. no.	Extract	Bacteria	Zone of Inhibition
1	Trikatu-Methanol extract	*S. aureus*	15 mm
2	Trikatu-Hexane Extract	*S. aureus*	Not detected
3	Trikatu-Aqueous Extract	*S. aureus*	Not detected
4	Positive control	*S. aureus*	40 mm

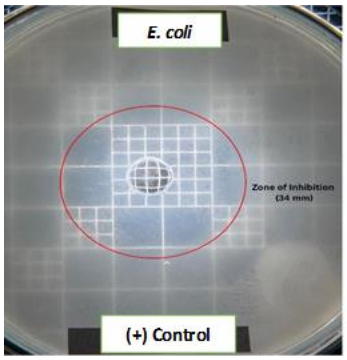
Table-2 ***Escherichia coli*** (Gram negative bacteria)

S. no.	Extract	Bacteria	Zone of Inhibition
1	Trikatu-Methanol extract	*E. coli*	16 mm
2	Trikatu-Hexane Extract	*E. coli*	Not detected
3	Trikatu-Aqueous Extract	*E. coli*	Not detected
4	Positive control	*E. coli*	34 mm









Result and Discussion

Chromatographic evaluation of Trikatu showed the presence of all its key bioactive markers, validating the authenticity of the formulation. The clear confirms good-quality raw materials and absence of major adulteration. This also indicates the presence of Piperine and Gingerols. Piperine is responsible for enhancing bioavailability and contributing to the pungency of the formulation, while gingerols play a key role in digestive activity. Their confirmed presence supports the pharmacological relevance and expected therapeutic effects of Trikatu.

Effectiveness of different extracts is determined by the size of the Growth inhibition zone (Diameter measured in mm) around the well prepared. In table 1 Trikatu-Methanol extract showed larger Inhibition zone against Staphylococcus aureus as compared to Trikatu Hexane and Aqueous extract. It showed the highest inhibition zone of 15 mm and positive control of 40 mm. In table 2 Trikatu- Methanol extract showed larger inhibition zone against Escherichia coli as compared to Trikatu Hexane and Aqueous extract. It showed the highest inhibition zone of 16 mm and positive control of 34 mm. These findings suggest that methanol is a more efficient solvent for extracting bioactive constituents responsible for antimicrobial activity in Trikatu.

Overall, the results indicate a correlation between chromatographic composition and antimicrobial performance. The confirmed presence of piperine and gingerols aligns with the observed antibacterial activity, as both compounds have documented inhibitory effects against pathogens. Thus, the study not only validates the authenticity of Trikatu but also highlights its potential as a natural antimicrobial agent, especially when extracted using methanol.

Conclusion

The study confirmed the authenticity and quality of Trikatu through HPTLC analysis, which showed the presence of key bioactive markers such as piperine and gingerols. These compounds support the traditional digestive and therapeutic properties. The antimicrobial evaluation demonstrated that the methanolic extract of Trikatu exhibited the strongest inhibitory activity against both Staphylococcus aureus and Escherichia coli, indicating that methanol is the most effective solvent for extracting antibacterial constituents. Overall, our study validates the purity of Trikatu and highlight its moderate antimicrobial potential, supporting its traditional medicinal use.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Aher, A. A. and Wahi, A. K. Pharmacological and phytochemical profile of Piper nigrum Linn.: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*; 2011; 3(1):1–5.
2. Balasubramani, S.P.; Venkata-subramanian, P. and Reddy, R. R. Phytochemical standardization of Trikatu—A polyherbal formulation using HPTLC. *Journal of Ayurveda and Integrative Medicine;* 2017; 8(2):84–91.
3. Borquaye, L.S. and Darko, G. Antimicrobial activities of Piper nigrum extracts. *Scientific African*.;

2020; 7:e00286.

4. Gupta, A.; Mahajan, S. and Sharma, R. Evaluation of antimicrobial activity of Piper longum L. *International Journal of Green Pharmacy*; 2013; 7(1):67–70.
5. Indian Pharmacopoeia Commission. High-Performance Thin Layer Chromatography (HPTLC) Guide-lines; 2020.
6. Kamatou, G. P. and Viljoen, A. M. Zingiber officinale: Phytochemical, pharmacological and toxicological overview. *Journal of Ethnopharmacology;* 2010; 131(2):289–302.
7. Kokate, C.K.; Purohit, A.P. and Gokhale, S.B. *Pharmacognosy*; 2018; 51st ed.; Nirali Prakashan.
8. Kulkarni, R. R.; Patki, P. S.; Jog, V. P.; Gandage, S. G. and Patwardhan, B. Efficacy of an Ayurvedic formulation Trikatu in respiratory diseases. *Journal of Ethnopharmacology*; 1998; 62 (2):137–142.
9. NCCLS. Performance Standards for Antimicrobial Disk Susceptibility Tests (7th ed.). *National Committee for Clinical Laboratory Standards*; 2002.
10. Patel, R. P. and Bhutani, K. K. Quality evaluation of Ayurvedic formulation using HPTLC. *Ancient Science of Life*; 2012; 31(4):153–157.
11. Rangari, V. D. Pharmacognosy and Phytochemistry (Vol. 2). Career Publications. 2009.
12. Sharma, P. V. Charaka Samhita (Vol. 1–4). Chaukhambha Orientalia. 2003.
13. Singh, G.; Kapoor, I.P.S.; Singh, P.; de Heluani, C. S.; de Lampasona, M. P. and Catalan, C.A. Chemistry and antimicrobial properties of ginger essential oil. *Food and Chemical Toxicology*; 2008; 46(10):3295–3302.
14. Sujatha, V. and Gopal, V. Antimicrobial activity of polyherbal formulation Trikatu. *World Journal of Pharmacy and Pharmaceutical Sciences,* 2017; 6(8):1450–1458.
15. WHO. Quality Control Methods for Herbal Materials. *World Health Organization;* 2011.