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# Preliminary Phytochemical Screening and Antibacterial Studies of Trikatu

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## A B S T R A C T

"Trikatu" is an Ayurvedic proprietary medicine. It is the mixture of fruits of *Piper nigrum*, *Piper longum* and rhizome of *Zingiber officinale*. This combination is found to be active against a wide range of health problems. The present study is to evaluate the phytochemical characterization and antibacterial activities of "Trikatu". The fruits of *P. nigrum*, *P. longum* and rhizome of *Z. officinale* were collected from local areas of Wayanad and Ernakulam districts of Kerala, India. Collected plant materials were washed and shade dried. The dried plant materials were then ground to fine powder and stored. Distilled water, methanol, ethanol, acetone and diethyl ether were used for extraction of the powdered samples. Analysis of phytochemicals were done using standard methods. The extracts showed the presence of flavonoids, alkaloids, phenols, tannins, quinine, cardiac glycosides, saponins, steroids, carbohydrates etc. The best results are obtained in methanol and ethanol extracts while diethyl ether extracts showed minimal presence of the compounds. Antibacterial activity was evaluated using agar well diffusion method against four strains of bacteria. Methanol and diethyl ether extracts showed maximum inhibitory activity against all test organisms.

***Key words*:** Phytochemistry, *Trikatu*, Antibacterial activity, *Piper nigrum*, *Piper longum*, *Zingiber officinale*

India has a rich and ancient heritage of traditional herbal medicine with different systems of medicinal practices which includes Ayurveda, Siddha and Unani medicine system. Traditional medicine is found to be very effective against some of the major health problems without any side effects. The development of these traditional medicines with the perspectives of safety, efficacy and quality will help to preserve the traditional heritage and to rationalize the use of natural products in health care. Herbal medicines are being used increasingly as dietary supplements to fight or prevent common disease. *Trikatu* is a combination of three herbs which have 'katu rasa' i.e., a pungent taste. It is the mixture of *P. nigrum*, *P. longum* and *Z. officinale*. *Trikatu* is also known as "three bitters". This combination maintains body metabolism and has a wide range of application in health problems like asthma, fever, cold, cough etc. Plants synthesize numerous bioactive compounds with antimicrobial, anti-inflammatory, antiviral properties etc. Reddy and Seetharam [1] studied the phytochemical properties of alcoholic extracts, antimicrobial and analgesic activities of *Trikatu churna* and its ingredients. Narasimha *et al.* [2] studied the anthelmintic activity of aqueous and ethanolic extract of *Trikatu churna* through phytochemical evaluation of crude extracts of the three ingredients.

Now a days, microbes are gaining resistance against drugs thus the need for invention of new therapeutic drugs are increasing. Dahikar *et al.* [3], Malvankar and Abhyankar [4] studied the *in vitro* antibacterial potential of *Trikatu churna* and its ingredients. Sunita *et al.* [5] studied the standardization of *Trikatu churna* using bioanalytical tools. The preliminary phytochemical screening showed the presence of alkaloids, tannins and essential oils, flavonoids, glycosides, and resins. A review on pharmacological and phytochemical properties of *Z. officinale* by Kumar *et al.* [6] documents the antimicrobial activity of the different organic extracts (n-hexane, ethyl acetate, ethanol and water) against *Coliform bacillus*, *Staphylococcus epidermidis* and *Streptococcus viridians*. Bhargava *et al.* [7] studied the chemical and phytochemical screening and evaluation of antimicrobial activity of *Z. officinale*. Phytochemical screening of ethanolic and methanolic plant extracts showed the presence of different phytochemicals. The ethanolic and methanolic extracts of *Z. officinale* showed strong antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*. Antibacterial activity of *P. nigrum* with special reference to its mode of action on bacteria was studied by Pavithra and Bhagya Lakshmi [8] and it was found that Gram positive bacteria were most susceptible than Gram negative bacteria. Aparna and Amit [9] studied the phytochemical and antimicrobial properties of *P. longum*. The

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antimicrobial assay was performed by three different methods. The well diffusion method of antimicrobial study showed that the alcoholic extract is active against both Gram positive and Gram-negative organisms. The present study is undertaken to evaluate the phytochemical characterization and antibacterial screening of the above three medicinal plants which together form "*Trikatu*".

## MATERIALS AND METHODS

The plant materials i.e., fruits of *Piper nigrum*, *Piper longum* and rhizomes of *Zingiber officinale* were collected separately from local areas of Wayanad and Ernakulam districts in the month of August 2019.

The collected fruits of *P. nigrum*, *P. longum* and rhizome of *Zingiber officinale* were washed and were shade dried. Samples were ground to fine powder separately. Five solvents namely diethyl ether, distilled water, acetone, ethanol and methanol were used for extraction. After 8 hours of shaking, the solvent extracts were filtered through Whatman No. 1 filter paper. The crude extracts were transferred to labelled sterile screw capped bottles and stored in refrigerator for further use. Preliminary phytochemical tests for the identification of alkaloids, carbohydrates, phenols, tannins, quinines, saponins, cardiac glycosides, coumarins, protein and amino acids were carried out using standard methods described by Harborne [10] and Khandelwal [11]. Antibacterial activity of the plant materials was evaluated by agar well diffusion method. The gram-positive bacteria *B. cereus* and *S. aureus* and gram-negative bacteria *Escherichia coli* and *V. harveyi* bacteria were selected. After incubation, zone of inhibition of bacteria were observed and measured [4].

## RESULTS AND DISCUSSION

Phytochemical extraction of *Zingiber officinale*, *P. nigrum* and *P. longum* were done using five solvents. Alkaloids were present in ethanol extracts of *Zingiber officinale* and *P. longum* whereas *P. nigrum* showed positive results in acetone and methanol. Carbohydrate was present in all the extracts. Test for phenols showed positive results in distilled water, ethanolic and methanolic extracts of *Zingiber officinale* and positive results were obtained in acetone, ethanol and methanol extracts of *P. nigrum* and *P. longum*. Flavonoids were present in all extracts of *Zingiber officinale* except aqueous extract. In the case of *P. nigrum* all the extracts showed absence of flavonoids and in *P. longum*, acetone and methanol showed presence of flavonoids. Ethanolic and methanolic extracts of *Zingiber officinale* and acetone, ethanol methanol extracts of *P. longum* showed the presence of tannins. In the case of *Zingiber officinale* all the extracts except acetone extract tested positive for terpenoids and steroids. In *P. nigrum* all extracts except diethyl ether extract showed presence of terpenoids while all extracts of *P. longum* showed positive results except distilled water. Saponins showed presence in all extracts except diethyl ether and the acetone in *P. nigrum*. Extract of *P. longum* showed positive results for saponins in distilled water, ethanol and methanol extracts. Amino acids were tested negative in all samples. Proteins were tested positive only in ethanol extract of *P. longum*. Cardiac glycosides were present in all the extracts (Table 1-3). The three samples showed antibacterial activity against all the bacteria tested. *E. coli* and *S. aureus* are found to be more sensitive against diethyl ether extracts of the *Trikatu churna* ingredients. *Zingiber officinale* showed higher inhibition zone in *S. aureus* (20mm) which is higher than all other extracts. Diethyl ether extract of *P. longum* showed a noticeable activity against *B. cereus*, *V. harveyi*, *E. coli* and *S. aureus* cultures with an inhibition zone of 10mm, 10mm, 12mm and 16mm respectively. Inhibition zone of diethyl ether extract of *Zingiber officinale* showed the lowest inhibitory activity against *V. harveyi* (6mm). The control did not show any significant inhibition zone.

Table 1 Phytochemical constituents of different extracts of *Zingiber officinale*

| Phytochemicals | Diethyl ether | Distilled water | Acetone | Ethanol | Methanol |
|---|---|---|---|---|---|
| Alkaloids | - | - | - | + | - |
| Carbohydrates | + | + | + | + | + |
| Phenols | - | + | - | + | + |
| Flavonoids | + | - | + | + | + |
| Tannins | - | - | - | + | + |
| Terpenoids | + | + | - | + | + |
| Saponins | - | + | + | + | + |
| Steroids | + | + | - | + | + |
| Amino acids | - | - | - | - | - |
| Protein | - | - | - | - | - |
| Glycosides | + | + | + | + | + |

Table 2 Phytochemical constituents of different extracts of *Piper nigrum*

| Phytochemicals | Diethyl ether | Distilled water | Acetone | Ethanol | Methanol |
|---|---|---|---|---|---|
| Alkaloids | - | - | + | + | - |
| Carbohydrates | + | + | + | + | + |
| Phenols | - | - | + | + | + |
| Flavonoids | - | - | - | - | - |
| Tannins | - | - | - | + | + |
| Terpenoids | - | + | + | + | + |
| Saponins | + | + | - | + | + |
| Steroids | - | + | + | + | + |
| Amino acids | - | - | - | - | - |
| Protein | - | - | - | - | - |
| Glycosides | + | + | + | + | + |

Table 3 Phytochemical constituents of different extracts of *Piper longum*

| Phytochemicals | Diethyl ether | Distilled water | Acetone | Ethanol | Methanol |
|---|---|---|---|---|---|
| Alkaloids | - | - | - | + | - |
| Carbohydrates | + | + | + | + | + |
| Phenols | - | - | + | + | + |
| Flavonoids | - | - | + | - | + |
| Tannins | - | - | + | + | + |
| Terpenoids | + | - | + | + | + |
| Saponins | - | + | - | + | + |
| Steroids | + | - | + | + | + |
| Amino acids | - | - | - | - | - |
| Protein | - | - | - | + | - |
| Glycosides | + | + | + | + | + |

Table 4 Antibacterial activity of Diethyl ether extracts

| Name of sample | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *B. cereus* | *V. harveyi* | *E. coli* | *S. aureus* |
| *Z. officinale* | 15 | 6 | 16 | 20 |
| *P. nigrum* | 12 | 12 | 12 | 11 |
| *P. longum* | 10 | 10 | 12 | |

Table 5 Antibacterial activity of aqueous extracts

| Name of sample | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *B. cereus* | *V. harveyi* | *E. coli* | *S. aureus* |
| *Z. officinale* | 6 | | 5 | |
| *P. nigrum* | 14 | 12 | 12 | 7 |
| *P. longum* | 9 | 15 | 10 | |

Table 6 Antibacterial activity of acetone extracts

| Name of sample | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *B. cereus* | *V. harveyi* | *E. coli* | *S. aureus* |
| *Z. officinale* | 6 | 9 | 5 | 10 |
| *P. nigrum* | 2 | 6 | 11 | 10 |
| *P. longum* | 9 | 6 | 9 | 12 |

Table 7 Antibacterial activity of methanol extracts

| Name of sample | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *B. cereus* | *V. harveyi* | *E. coli* | *S. aureus* |
| *Z. officinale* | 10 | 8 | 8 | 19 |
| *P. nigrum* | 9 | 10 | 7 | 9 |
| *P. longum* | 10 | 14 | 10 | 14 |

Table 7 Antibacterial activity of ethanol extracts

| Name of sample | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *B. cereus* | *V. harveyi* | *E. coli* | *S. aureus* |
| *Z. officinale* | 10 | | 16 | 10 |
| *P. nigrum* | 12 | 5 | 12 | 5 |
| *P. longum* | 9 | 7 | 11 | 11 |

Aqueous extract shows lower activity when compared to other solvent extracts. Aqueous extract of *P. nigrum* exhibited strong inhibition activity against all the test organisms but the maximum inhibitory activity was showed by extract of *P. longum* against *V. harveyi* (15mm). Aqueous extract of *Z. officinale* showed lowest activity against all bacteria. *V. harveyi* and *S. aureus* showed resistance to *Z. officinale* extracts. The control did not show any significant inhibition zone.

Acetone extract exhibited maximum activity against *S. aureus* and *P. longum* showed highest activity against *S. aureus* (12mm) and *P. nigrum* in *B. cereus* showed the lowest inhibition zone (2mm). The inhibition zone of acetone extract of *Z. officinale*, *P. nigrum* and *P. longum* against *S. aureus* are 10mm, 10mm and 12mm respectively. Compared to other extracts aqueous extract of *P. nigrum* in *B. cereus* showed the lowest antibacterial activity.

Methanol extracts of the three plant materials showed good activity against all bacteria tested. These extracts showed maximum activity against *S. aureus* (19mm) and produced noticeable zone of inhibition against rest of the test organisms. Ethanol extract showed maximum activity in *E. coli* and minimum activity in *V. harveyi*.

In Ayurveda the fruits of *P. longum, P. nigrum* and the rhizome of *Z. officinale* collectively in equal proportion called as "*Trikatu*", a sanskrit word meaning three acids. The ancient documented Ayurvedic Materia Medica which dates back to

6000 years B.C. mentions these three herbs are essential ingredients of numerous prescriptions and formulations used for a wide range of disorders [12]. The present study aims to analyze the phytochemical and antibacterial potential of "*Trikatu"*.

In preliminary phytochemical screening, diethyl ether extract showed the presence of carbohydrates, flavonoids, terpenoids, steroids, saponins and glycosides in the extract. Aqueous extract of the three ingredients showed the presence of carbohydrates, phenols, Terpenoids, steroids, saponins and glycosides. The preliminary phytochemical observations of crude extracts of four different test samples of *Trikatu* and its plant ingredients have shown the occurrence of alkaloids, Flavonoids, tannins, lignin and steroids [3]. Acetone extract showed the presence of alkaloids, carbohydrates, phenols, terpenoids, saponins, tannins, steroids and glycosides, in all the samples in phytochemical analysis. The ethanol extract showed the presence of all the compounds except amino acids. Presence of carbohydrates, phenols, tannins, terpenoids, saponins and steroids are identified in the methanol extracts of the three ingredients. Carbohydrates and glycosides are present in the all the extracts of *Z. officinale*, *P. nigrum* and *P. longum*. In the present study steroid was detected in *P. nigrum* but steroids were not identified [1]. Almost all bioactive compounds were detected in the study except for proteins and amino acids.

In the present investigation, the antibacterial activity of *Z. officinale*, *P. nigrum* and *P. longum* against four strains of bacteria were tested. The ingredients of *Trikatu* showed varying levels of inhibition against the growth of tested bacterial strains in each extract. In the present study, diethyl ether and methanol extract of *Z. officinale* showed highest activity against bacterial strains when compared to other extracts in distilled water, acetone, ethanol. Dahikar *et al.* [3] while working in the antibacterial potential in *Trikatu*, observed that acetone extract of *P. nigrum* had strong antibacterial activity against *S. epidermidis* and *S. aureus*, while moderate activity was observed against *P. vulgaris*, *S. typhi*, *E. coli* etc. The distilled water extract of *Z. officinale* showed mild activity against *B. cereus* and the acetone extract showed moderate activity against *E. coli* and *B. cereus*. *Z. officinale* has been showed possess antimicrobial activity in various pathogens [13]. Acetone extract of *P. nigrum* showed minimal activity against *B. cereus* and *V. harveyi*. *P. longum* have being reported as a strong antibacterial agent against *B. cereus* and *E. coli* [14], which is traditionally used for chronic bronchitis, asthma [15]. Ethanol extract of *P. nigrum* showed mild activity against *V. harveyi*. *S. aureus* and *V. harveyi* showed resistance in distilled water extract of *Z. officinale*. *S. aureus* also exhibited resistance to distilled water extract of *P. longum*. Among the three ingredients of *Trikatu* examined during the study the diethyl ether and methanol extract of *Z. officinale* has higher antibacterial activity against *S. aureus*. Acetone extract of *P. nigrum* showed lowest antibacterial activity against *B. cereus*. Therefore, this study provides the preliminary scientific evidence for use of *Trikatu* for enteric bacterial infection.

## CONCLUSION

The analysis of phytochemicals in the present study has proved that fruits of Piper nigrum, Piper longum and rhizome of Zingiber officinale showed the presence of the phytochemicals which are known as biologically active compounds such as phenols, flavonoids, saponins, tannins and alkaloids. So, these plants could be used as a potential source of bioactive compounds. Methanol extracts of all the three plant materials showed good activity against all bacteria tested. Therefore, this study provides the preliminary scientific evidence for use of Trikatu for enteric bacterial infection.

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