Phytochemical analysis and antibacterial activity of methanolic extracts of roots of piper longum

Bharvi Mor, Akhila Nair, Hetal Parmar, Neha Jangbari, Archana Rana, *Usmangani Tabani

Department of Microbiology, Christ College, Rajkot
*tabaniusmangani@gmail.com

Abstract:

Phytochemicals present in plants possess various biological activities including antimicrobial activities. *Piper longum* has been used for centuries to cure various ailments. Methanolic extract of roots of *Piper longum* contains phytochemicals that are antibacterial in nature. Glycosides, Flavonoids, Tannins, Alkaloids, and Terpenoids are present in methanolic extract of roots of Piper longum. The zone of Inhibition of methanolic extract was found to be 8mm against *Escherichia coli*, 9mm against *Staphylococcus aureus*, 7mm against *Shigella flexneri*, 10mm against *Salmonella enterica typhimurium,* and 12mm against *Bacillus megaterium*. The present study suggests that the methanolic extract of the roots of *Piper longum* can be used as an antibacterial agent against selected pathogens.

Introduction

Plant-based traditional medicine plays an important role in the development and progress of modern research by serving as a starting point for developing novelties in pharmaceutical research [1]. From traditional medicinal plants, substances have been used to create a variety of modern medicines [2]. WHO estimates that around 80% of the world's population relies on medicinal plants as a primary source of health [3], increasing demand in developing countries [4]. Even in developed countries, traditional plant-based medicines, often referred to as complementary and alternative medicine (CAM), are steadily increasing in use [5]. Medicinal plants show great potential for developing new drug molecules for various serious diseases.

Due to the presence of important bioactive compounds and secondary metabolites, *Piper longum* has attracted worldwide attention for its various therapeutic uses. Major reported pharmaceutical activities include antitumor, antioxidant, immunomodulatory, antifertility, and anti-insect activity.

Piper longum is not only used as a spice to flavor foods but is also widely used in various traditional medical practices around

the world in traditional Indian medicine. The importance of *Piper longum* is documented in ancient texts such as Charaka Samhita, Sushrut Samhita, and Vagbhata Astangya Hardaym. All parts of the plant, including stems, roots, leaves, and fruits, are reportedly used to treat many ailments. Piper Longum is an important part of the Ayurvedic, Siddha, and Unani medical systems and is used to treat a variety of ailments. The berries and roots are used in over 100 raw medicinal preparations, including asthma, bronchitis, colic (School), catarrhal fever (Jwar), and liver and spleen diseases. (Preha), hemorrhoids (Arsha), urinary tract disease (Pramé), leprosy (Kushta), rheumatism (Amavata), gastritis, indigestion, menstrual disorders, bleeding during childbirth, anorexia, gout, paralysis, epilepsy, snake bites, Scorpion antidote bite. [6,7, 8].

In this study antibacterial study of methanolic extract of roots of *Piper longum* suggests that it can be used to treat diseases caused by the above-selected bacteria.

Materials and methods

Collection of Sample

Piper longum powder was procured from the Ayurvedic medical store.

Extraction preparation Method of *Tridax procumbens* and P*iper longum*

10 g of dried powder was soaked in 200 ml of methanol. The flask was then covered with cotton and placed on a rotary shaker at 37°C for 72 hours. The extract was filtered through Whatman No. 1 filter paper and the filtered extract was evaporated at room temperature [9]. A stock solution of 100 mg/ml of extract is prepared in dimethyl sulfoxide (DMSO) as it does not inhibit bacterial growth [10]

Phytochemical Test

1. Tannin/ polyphenol test

Addition of 3 drops of $FeCl_3$ to the diluted extract colored the solution blue due to gallic tannins and green due to the presence of catechol tannins [11].

2. Glycoside test

Molisch reagent test: 5 mL of Molisch reagent and concentrated sulfuric acid were added to the extract. The purple color indicated glycosides [12].

3. Flavonoid Test: Shinoda Test

4 ml of extract, 1.5 ml of 50% methanol solution, and a small lump of magnesium were heated. A red color was observed with flavonoids when 5-6 drops of concentrated HCl were added [11].

4. Terpenoids test

0.2 g of each sample in 2 ml chloroform, 3 ml concentrated H_2SO_4. A reddish-brown color indicated the presence of terpenoids [12].

5. Alkaloid Test: Meyer Test

1 ml of Meyer's reagent was added to 2 ml of extract. The presence of a pale-yellow precipitate indicated the presence of alkaloids [11].

6. Saponin test

A 2 g powder sample was boiled in 20 ml of distilled water. 10 ml of the filtrate and 5 ml of distilled water were vigorously shaken. Due to the presence of foam, it can conclude that Saponin is present [12].

7. Volatile Oil Test

2 mL of extract was shaken with 0.1 mL of NaOH and a small amount of dilute hydrochloric acid. A white precipitate indicated the presence of volatile oils [11].

8. Cardiac glycoside test

5 mL of plant extract was treated with 2 mL of glacial acetic acid containing 1 drop of FeCl3 solution. Purple rings may appear or greenish rings may form which show the presence of cardiac glycosides [11].

9. Steroid Test

1 g botanical extract was dissolved in a few drops of acetic acid and 1 drop of concentrate H_2SO_4 was added. A green appearance indicated the presence of steroids.[11]

Antibacterial Analysis

A colony of pathogenic bacteria (*Escherichia coli, Salmonella enterica typhimurium, Staphylococcus aureus, Bacillus megaterium, and Shigella flexneri*) was taken with the help of a sterile wire loop and inoculated in the Nutrient broth under the aseptic condition. Incubation was carried out in the incubator at 37ºC for 24 hrs. The antimicrobial activity of the crude extract was determined by the agar well diffusion method. The crude extract was dissolved in DMSO. The microbial culture was diluted to 0.5 MacFarland unit by sterile N-saline (0.9%w/v). The test organisms were spread on a Muller Hinton agar plate. Wells of 8 mm diameter were punched into the agar medium and filled with 100 µl methanolic extract of roots of *Piper longum* with DMSO as a control. All plates were incubated at 37ºC for 24 hours. The antimicrobial activity was evaluated by measuring the zone of inhibition [13,14].

Results

Phytochemical analysis of *Piper longum*:

Table 1: Phytochemical analysis of the crude methanol extract.

No.	Phytochemical test	Observation	Result
1.	Steroid	Green color appeared	-
2.	Glycosides	Violet color formed	+
3.	Flavonoids	Yellow color formed	+
4.	Saponins	Foam not formed	-
5.	Tannins	Solution turned green	+
6.	Alkaloids	Blue color observed	+
7.	Volatile	White ppt appeared	-
8.	Cardiac	Violet or Greenish ring formed	-
9.	Terpenoids	Reddish brown color formed	+

Note: (+) means phytochemical is present,
(-) means phytochemical is absent

Fig. 1: Phytochemical Analysis of methanolic extract of *Piper longum*

The result of the phytochemical analysis of the methanolic extract *of Piper longum* is tabulated in **Table 1**. **Fig. 1** depicts the presence of tannin, glycoside, flavonoid, alkaloid, and terpenoids. The presence of these compounds may confer antibacterial activity on the methanolic extract of *Piper longum* roots.

Antimicrobial Analysis of *Piper longum*

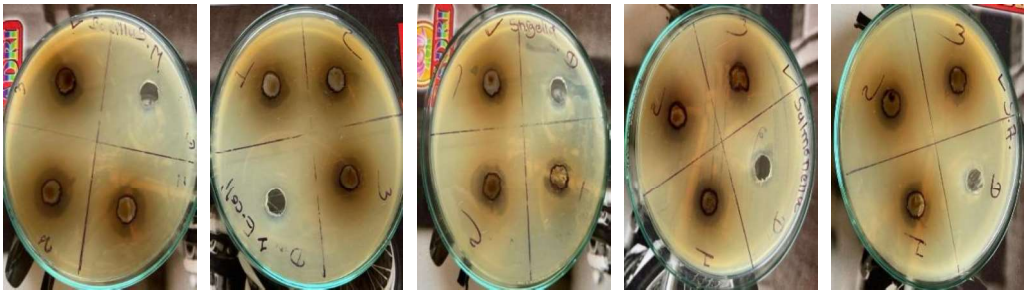

Fig. 2: Zone of inhibition of *Piper longum* plant extract against *Escherichia coli, Bacillus megaterium, Shigella flexneri, Salmonella enterica typhimurium, and Staphylococcus aureus* respectively.

Table 2: *Piper longum* extract showed antimicrobial activity against *Escherichia coli, Bacillus megaterium, Shigella flexneri, Salmonella enterica typhimurium, and Staphylococcus aureus*

No.	Microorganism	Mean Zone of inhibition (in mm)	Dimethyl sulfoxide as Control
1.	*Escherichia coli*	8mm	-
2.	*Staphylococcus aureus*	9mm	-
3.	*Shigella flexneri*	7mm	-
4.	*Salmonella enterica typhimurium*	10mm	-
5.	*Bacillus megaterium*	12mm	-

The zone of inhibition as obtained by the agar well diffusion method is shown in **Fig. 2**. The data of the results is tabulated in **Table 2**. The result showed that methanolic extract of roots of *Piper longum* has antimicrobial activity against *Escherichia coli, Bacillus megaterium,*

Shigella flexneri, Salmonella enterica typhimurium, and Staphylococcus aureus. Maximum inhibition was found against *Bacillus megaterium* (12mm) and least against *Shigella flexneri* (7mm).

Discussion

Phytochemical analysis of the methanolic extract of *Piper longum* showed the presence of Glycoside, Flavonoid, Tannin, Alkaloid, and Terpenoid that may contribute antimicrobial properties to the extract against various microorganisms. *Piper longum* extracts exhibit significant antibacterial activity when tested against different bacterial pathogens such as *Staphylococcus albus, Pseudomonas aeruginosa, Escherichia coli, Bacillus megaterium,* and *Salmonella typhi* [15]. The current study showed that methanolic extracts of roots of *Piper longum* exhibited antibacterial activity against *Escherichia coli, Salmonella enterica typhimurium, Staphylococcus aureus, Shigella flexneri, and Bacillus megaterium*.

Conclusion

Phytochemicals present in the methanolic extract of roots of *Piper longum* and results of antibacterial activity deduced that these extracts are antibacterial in nature.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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