



Antibacterial Activity of *Piper nigrum* Leaf against Different Species of Pathogenic Microbes

Reshmi R. P*, S. Justin Raj**

* PG, Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliyakavilai, India.

** Assistant Professor and Research Guide, Department of Biotechnology, Malankara Catholic College, Mariagiri, kaliyakavilai, India.

*Corresponding author's E-mail: rajstephy6@gmail.com

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ABSTRACT

Piper nigrum is a natural medicinal plant used to treat many diseases. It is used for various medicinal purposes in traditional herbal medication. The leaf of *Piper nigrum* has phytochemical compounds like alkaloids, tannins, phenolics etc. Medicinal plants represent a rich source of antimicrobial agents and medicinally used in different countries as source of many potent and powerful drugs. Piperine is the major chemical constituent responsible for the bitter taste of the black pepper. Recent scientific research has established the presence of many active compounds in these spices that are known to possess specific pharmacological properties. It is also used in foods and cosmetic purposes due to their vast pharmacological potential. The present study evaluated the role of antibacterial activity of five different leaf extracts in four different pathogens. Among them *Shigella boydii* showed highest activity against methanol leaf extract using disc diffusion method.

Keywords: Tannins, Pepper, Piperine, Antibiotics, Cosmetic.

INTRODUCTION

Phytochemicals are non-nutritive components present in a plant-based diet that exert protective or disease-preventing effects.¹ Carbohydrates are a common source of energy in living organisms. Plant contains a variety of chemically active compounds such as flavonoid, terpenoids, alkaloids, coumarin, saponin, polysines, sulphides and furyl compounds. Phytochemicals provide colors to plants and an array of flavors both pleasant and unpleasant when consumed. They contain active constituents that are used in the treatment of many human diseases.² They are effective in the treatment of infectious diseases, while simultaneously alleviating many of the side effects that are often connected with synthetic antimicrobial.³ *Piper nigrum* sometimes called Indian Long pepper, is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. The fruits have a bitter, hot, sharp taste, tonic to the liver, stomachic, abortifacient, aphrodisiac and digestive. *Piper* plants are also known under the common name "pepper".⁴ One of the most extensively studied compounds isolated from *Piper* plant is piper longumine, also known as piplartine. Piplartine is an amide alkaloid found in several *Piper* species (Piperaceae). It has been shown that piplartine has potential anticancer properties.⁵ The *Piper nigrum* contains some of the antimicrobial components such as Terpinene, α -pinene, β -pinene, Linaleol and Terpeneol.⁶ Piperine, pipene, piperamide and piperamine possess diverse pharmacological activities. Piperine has been found to enhance the therapeutic efficacy of many drugs, vaccines and nutrients by increasing oral bioavailability by inhibiting various metabolising enzymes⁷ and there is preclinical evidence that it may have modest

immune system enhancing properties.⁸ Medicinal plants are considered new resources for producing agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections.⁹ Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens.¹⁰ The present study evaluate the antimicrobial effect of five different *Piper nigrum* leaf extract against four different pathogens such as *Shijiella boydii*, *Klebsilla pneumonia*, *Staphylococcus aureus* and *Flacobacter*.

Taxonomy

Kingdom : Plantae
Class : Equisetopsida
Sub class : Magnoliidae
Order : Piperales
Family : Piperaceae
Genus : *Piper*
Species : *nigrum*



Piper nigrum



MATERIALS AND METHODS

Plant Material

Fresh leaves of black pepper (*Piper nigrum*) were collected from local area, Trivandrum district in a plastic bag and cleaned with distilled water.

Extract Preparation

For each extract preparation, fresh leaves of 20gm were weighed and grinded with 100ml of chloroform, ethyl acetate, acetone and ethanol. Then the solutions were centrifuged at 5000rpm for 15 minutes, supernatant were collected and filtered through whatman number 1 filter paper.

Disc diffusion method

The antibacterial activity of *Piper nigrum* leaf was evaluated by using disc diffusion method. Lawns of each organism were prepared on nutrient agar medium (20ml). The plant extract was concentrated by evaporation and was added to sterile filter discs (size 3mm) and allowed the solvent to evaporate after each addition. The discs were then placed on air dried surface of the medium. The plates were then incubated for 24hrs at 37°C. After incubation the degree of sensitivity were determined by measuring the zone of inhibition of growth around the disc.¹¹

Bacterial cultures

The microorganisms (Gram positive and Gram negative) were obtained from Microbial Type Culture Collection (MTCC), institute of microbial technology, Chandigarh. The bacterial cultures were maintained in nutrient broth as turbid growth and kept in refrigerator.

RESULTS

In the present study the antibacterial effect of *piper nigrum* leaf extracts were tabulated in table (1) and presented in figure (1). *Piper nigrum* leaf extracts showed antibacterial activity against both gram negative and gram positive bacteria with zone of inhibition ranged from 0.1 cm to 1 cm. The chloroform leaf extract of *Piper nigrum* exhibited greater antimicrobial activity against the selected bacterial strains. The results showed that methanol leaf extract of *Piper nigrum* showed highest activity (0.8 cm) against *Shigella boydii*. The chloroform and ethyl acetate leaf extract of *Piper nigrum* showed 0.4 cm inhibition zone against *Shigella boydi* and the results were plated in plate A and B.

Plate (A,B) shows the antibacterial activity of various leaf extract against *Shigella boydii*



A

B

Plate (C,D) shows the antibacterial activity of various leaf extract against *Klebsiella pneumonia*



C

D

Plate (E,F) shows the antibacterial activity of various leaf extract against *Staphylococcus aureus*

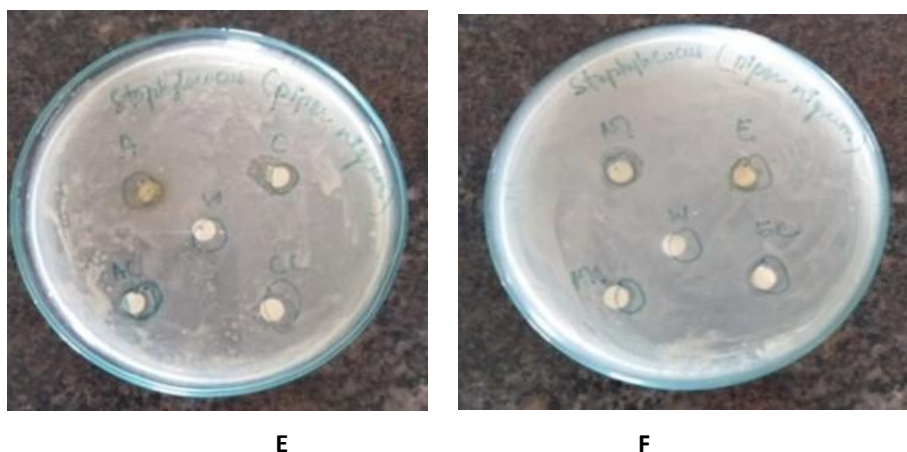


Plate (G,H) shows the antibacterial activity of various leaf extract against *Flacobacter*

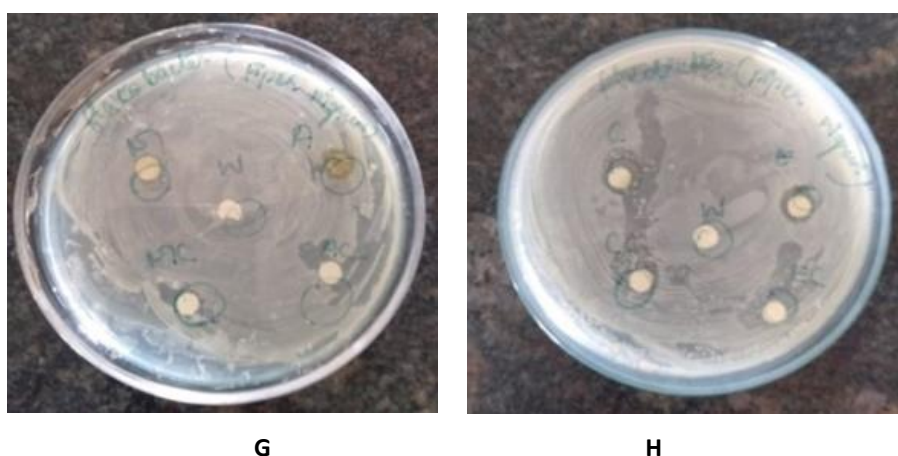


Table 1: The antibacterial activity of ethyl acetate, acetone, chloroform and methanol, leaf extracts of *Piper nigrum* against pathogenic microbes.

Sl. No	Pathogens	Zone of Inhibition (cm)				
		Ethyl acetate Leaf extract	Acetone Leaf extract	Chloroform Leaf extract	Methanol Leaf extract	Control
1	<i>Shijiella boydii</i>	0.4	0	0.4	0.8	–
2	<i>Klebsilla pneumonia</i>	0.2	0.1	1	0.2	–
3	<i>Staphylococcus aureus</i>	0.4	0.5	0.3	0.4	–
4	<i>Flacobacter</i>	0.4	0.5	1	0.6	–

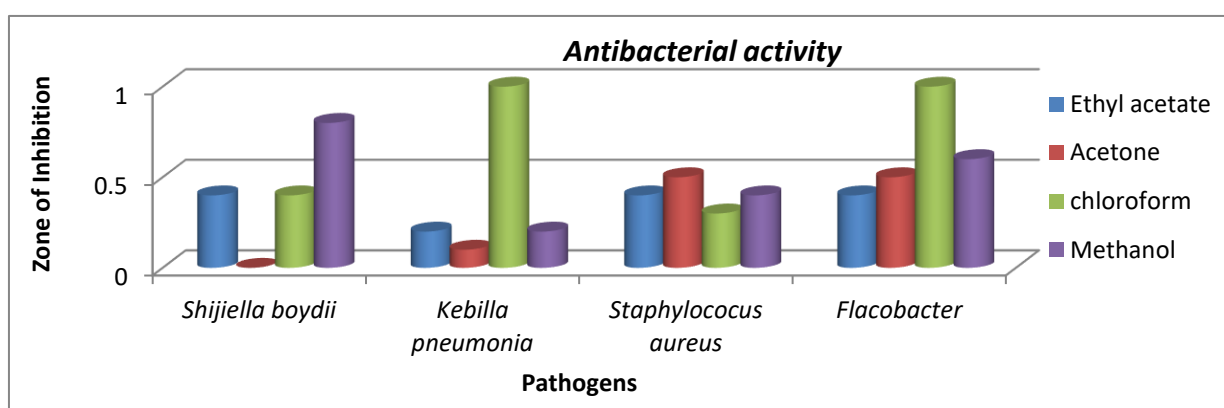


Figure 1: The antibacterial activity of ethyl acetate, acetone, chloroform and methanol leaf extracts of *Piper nigrum* against pathogenic microbes.

The chloroform leaf extract showed highest activity (1cm) against *Klebsiella pneumonia*. Ethyl acetate and methanol leaf extract showed 0.4 cm inhibition zone in *Staphylococcus aureus* and 0.2 cm inhibition zone against *Klebsiella pneumonia* followed by 0.1 cm inhibition zone in acetone leaf extract, the results were plated in plate C and D. Acetone leaf extract shows 0.5 cm inhibition and chloroform leaf extract shows 0.3 cm inhibition zone against *Staphylococcus aureus* (plate E and F). The Chloroform leaf extract shows 1cm inhibition zone against *flacobacter* whereas 0.6 cm in methanol extract, 0.5 cm inhibition zone in acetone leaf extract and the results were plated in plate G and H. Among the tested *Piper nigrum* leaf extracts the chloroform leaf extract showed highest activity against two pathogens (*klebsilla and flacobacter*) and the other leaf extracts showed poor antibacterial activity against *Klebsilla pneumonia*.

DISCUSSION

Due to the side effects and bacterial resistance against the antibiotics, the scientist developed new drugs from natural sources such as plants, which have been extensively used as alternative treatment for disease.^{12,13} Several studies reported that phytochemicals like terpenoids, flavonoids, tannins, alkaloids, steroids and some phenolic compounds are responsible for the antibacterial activity of the plant extract.^{14,15} The mode of action of antimicrobial agents also depends on the type of microorganisms and is mainly related to their cell wall structure and the outer membrane arrangement. This study confirms that the leaf extracts of *Piper nigrum* possess antibacterial activities. In the present study, the highest inhibition zone was determined by chloroform extract on two bacteria (Table 1). The least activity was shown in *Klebsilla pneumonia*. The antibacterial activity of chloroform leaf extract possess antibacterial activity due to the presence of phytochemical compounds like alkaloids and flavanoids.¹⁶ The results are in correlation with the results of Mital patai *et al*, in this study ethanol extract are highly active towards the tested bacterial strain and its inhibition zone are similar to streptomycin.¹⁷ While screening medicinal plants for antibacterial activity, it is generally expected that a greater number of compounds would be active against Gram positive rather than Gram negative bacteria.¹⁸ Pepper, ethanol and aqueous extracts were analyzed by Shamsuddeen *et al*. and it was found to contain phytochemicals like alkaloids, flavonoids, saponins, reducing sugars in ethanol and aqueous extracts and lack steroids and tannins.¹⁹ According to Ram Kumar Pundir *et al*. the antimicrobial activity of black pepper is due to the presence of essential oil (3%), whose aroma is dominated by monoterpenes hydrocarbons: sabinene, β -pinene and limonene.²⁰ Studies reported that piperine present in black pepper has antimicrobial activity.²¹ Spices we used in our daily diet can provide protection towards bacteria.²²

CONCLUSION

The results of the present study insights the possibility of novel drugs from this plant. The *Piper nigrum* contain constituents which may be useful for various herbal

formulation as anti-inflammatory, analgesic and antiasthmatic. It was revealed from this study that Black Pepper possesses significant antibacterial potential for the treatment of different ailments and diseases and found to be safe. Piperine exhibited wide spectrum therapeutic potential and also emerged as an excellent adjuvant to enhance the therapeutic efficacy of the concurrently administered drugs and nutrients. Further detailed research studies are needed to obtain more scientific data on this miraculous King of spices.

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