



***In vitro* Antibacterial Activity of *Piper Longum* L. Fruit**

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ABSTRACT

In this study the attempt was made to evaluate the antimicrobial activity of various solvent extracts of fruit of *Piper longum* L. against different gram positive and gram negative bacteria by using disk diffusion method. The petroleum ether extract was resistant towards all the tested bacterial strains while ethyl acetate was highly active. Among all the gram positive bacteria *Staphylococcus aureus* was highly sensitive with inhibition zone 24.33mm in presence of 500mg/ml ethyl acetate extract while in case of gram negative bacterial strains *Pseudomonas aeruginosa* and *Vibrio cholerae* were highly sensitive with inhibition zone 22.66mm. Hexane extract was least inhibitory towards all the bacterial strains.

Keywords: Antibacterial activity, ethyl acetate extract, *Piper longum*.

INTRODUCTION

The medicinal plant products and their active constituents played an important role to treat various diseases. Natural products, such as those found in plants, or their semi-synthetic derivatives, may provide novel compounds that can be developed into antimicrobial drugs^{1, 2}. The developing countries dependent on traditional medicine for variety of diseases³. In the last two decades, there has been increased interest in investigation of natural products as source of new antibacterial agents. Several experimental studies have contributed scientific evidence for the pharmacological effects of medicinal plants observed in folk medicine⁴. Antimicrobial screening and phytochemical investigations of plants from diverse region of the world have established the presence of antimicrobial activities and novel antimicrobial compounds in many species⁵⁻¹⁰.

The dried fruit of one such plant *Piper longum* Linn (Piperaceae), commonly known as Indian Long pepper, used as a spice and seasoning, is known to possess multitude of pharmacological activities. The fruits and roots are attributed with numerous medicinal uses, and may be used for diseases of respiratory tract, viz. cough, bronchitis, asthma also as anti-irritant and analgesic. The fruits have used as liver tonic, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive¹¹. However, root and stem are used as an important drug in the Ayurvedic and Unani systems¹². Review of literature revealed that the all plant parts of *P. longum* are very useful and the fact that few reports are available on the antibacterial activity of *P. longum* fruit extract¹³⁻¹⁵. The present work was aimed to evaluate the antibacterial property of different organic solvent extracts of *P. longum* fruit on some selected pathogenic bacterial strains.

MATERIALS AND METHOD

Plant materials

The fruits of *P. longum* L. were bought from a local herbal market, and the identity of the fruit was authenticated at the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University (B.H.U.), Varanasi.

Preparation of extracts

The collected fruits were washed thrice with distilled water and dried on blotting paper in laboratory at (37 ± 1)⁰C for 24 hours. After drying, the fruits were ground in grinding machine. Dry powdered plant fruits were extracted separately in methanol, ethyl acetate chloroform, hexane, petroleum ether to obtain their extracts. Extracts were filtered by filter paper (Whatmann No.1), concentrated and dried under reduced pressure in a rotary evaporator. All the dried extracts were stored in airtight vials at 4°C for subsequent use.

Test organisms

The bacterial strains *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Bacillus megaterium*, and four Gram negative *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Shigella dysenteriae*, *Salmonella typhi* were obtained from the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Screening for anti-bacterial activity

Sensitivity of bacterial strains towards different solvent extracts of fruits of *P. longum* was assayed by using the modified Kirby Bauer Disk Diffusion susceptibility method¹⁶. The bacterial strain (4-5 colonies) to be tested was suspended in 4ml of normal saline (0.85 %) and the density of suspension adjusted to approximately 10⁸ CFU ml⁻¹ using a 0.5 M barium sulphate suspension as the



standard turbidity. The surface of the sterile 3.8% Mueller Hinton Agar (Hi-Media) in Petri dishes was dried and the test strain was inoculated with a sterile swab to obtain a bacterial lawn. Sterilized discs were placed on the agar and 12µl of fruit extracts was placed directly on the disk to determine the inhibition zone. After incubation for 18 hours at 37°C the diameter of the inhibition zone was measured. DMSO was taken as control for organic extracts.

RESULTS AND DISCUSSION

Four gram positive and four gram negative bacterial strains were screened for sensitivity against different organic solvent extracts. All the tested gram positive and gram negative bacterial strains were resistant towards petroleum ether extract while highly sensitive towards ethyl acetate extract at 500mg/ml concentration.

Among all the gram positive bacterial strains *S. aureus* showed maximum sensitivity with inhibition zone

24.33mm (table 1) while among gram negative bacterial strains *P. aeruginosa* and *V. cholerae* were highly sensitive showed maximum inhibition zone 22.66mm in presence of 500mg/ml concentration of ethyl acetate extract (table 2). Successful prediction of botanical compounds from *P. longum* is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioner use water primarily as a solvent, but our studies showed that ethyl acetate extract of the plant was much better and effective. This may be due to the better solubility of the active components in organic solvent as chloroform, ethyl acetate, methanol¹⁷⁻¹⁹. These observations can be rationalized in terms of the polarity of the compound being extracted by each solvent and in addition to their intrinsic bioactivity by their ability to dissolve or diffuse in the different media used in the assay.

Table 1: Sensitivity of the different gram positive bacterial strains to the various extracts (500 mg/ml) of the fruit of *Piper longum* L.

Bacterial strains	Solvent extract (500mg/ml)									
	Petroleum ether		Hexane		Chloroform		Ethyl acetate		Methanol	
	Zone of inhibition (mm) (mean±SD)									
<i>S. aureus</i>	0.00	–	10.66±1.52	++	20.33±2.08	+++	24.33±1.52	+++	16.66±1.52	+++
<i>E. faecalis</i>	0.00	–	12.66± 2.08	++	19.00±2.64	+++	21.66±1.52	+++	21.00±1.00	+++
<i>S. pneumoniae</i>	0.00	–	13.33±1.15	++	18.33±0.57	+++	21.00±1.00	+++	15.00±1.00	++
<i>B. megatarium</i>	0.00	–	11.66±0.57	++	16.00±1.00	+++	22.00±1.00	+++	17.33±1.15	+++

(–) no susceptibility, (+) susceptible (inhibition zone ≤ 10mm), (++) inhibition zone between 10-15 mm, (+++) inhibition zone > 15 mm

Table 2: Sensitivity of the different gram negative bacterial strains to the various extracts (500 mg/ml) of the fruit of *Piper longum* L.

Bacterial strains	Solvent extract (500mg/ml)									
	Petroleum ether		Hexane		Chloroform		Ethyl acetate		Methanol	
	Zone of inhibition (mm) (mean±SD)									
<i>E.coli</i>	0.00	–	6.33±1.52	+	11.33±1.15	++	14.33±0.57	++	9.33±1.15	+
<i>P. aeruginosa</i>	0.00	–	12.00±1.00	++	20.66±1.52	+++	22.66±1.52	+++	15.66±0.57	+++
<i>S.typhi</i>	0.00	–	10.33±0.57	++	11.66±1.52	++	19.33±1.15	+++	10.66±0.57	++
<i>V. cholerae</i>	0.00	–	10.00±1.00	+	10.00±1.00	+	22.66±1.15	+++	15.00±1.00	++

(–) no susceptibility, (+) susceptible (inhibition zone ≤ 10mm), (++) inhibition zone between 10-15 mm, (+++) inhibition zone > 15 mm

Gram-positive bacteria were more susceptible because they have only outer peptidoglycan layer which is not an effective barrier²⁰. The gram-negative bacteria have outer phospholipidic membrane that make cell wall impermeable to lipophilic solutes, while the porins constitute a selective barrier to hydrophilic solutes.

P. longum, therefore, can be promising sources of antimicrobial agents. It can be used to specific bacteria, which are resistant to commonly used antibiotics, beside their use as traditional spice. The results of the study provides basis for subsequent bioactivity- guided fractionation of the extracts of *P. longum* fruit and isolation of active compound.

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Ms Chandan has done Ph.D on the topic "*Experimental evaluation of effect of Piper longum fruit on Mycobacterium species*" from Banaras Hindu University, Varanasi. She is having one year teaching experience at RGSC, BHU, Barkacha.