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## SYNERGISTIC EFFECT OF PIPER LONGUM EXTRACT WITH CIPROFLOXACIN ON MRSA

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### ABSTRACT

*Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become global concern. The clinical efficacy of many existing antibiotics has being threatened by the emergence of multidrug resistant pathogens. Piper longum, an alkaloidal rich plant belongs to the family Piperaceae, is rich in Piperine which is characterized both qualitatively by phytochemical analysis and through quantitative estimation. Piperine, an isomer of 1-piperoyl-piperidine present in the diethyl extract of fruit and Root of piper longum along with Ciprofloxacin showed good synergistic activity against Methicillin Resistant Staphylococcus aureus by enhanced accumulation and decreased efflux of antibiotics from bacterial cells.*

**Key words:** *Piper longum, Piperine, MRSA, Ciprofloxacin*

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### INTRODUCTION

*Piper longum* (long pepper), sometimes called as Indian long pepper, is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as spice and has generally hotter taste. Black pepper and long pepper have been used medicinally used for centuries. In recent years, extensive research data on phytochemistry and unique pharmacological actions of these plants have also become available. The material medica of Ayurveda, which dates back to 6000 B.C, has many references and advocating the use of pepper in a

variety of ailments, particularly those pertaining to the gastrointestinal tract (Chahal et al., 2011).

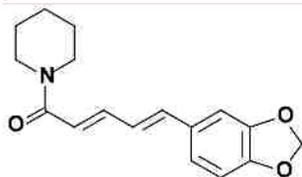
### Taxonomic classification

Kingdom	-	Plantae
Division	-	Magnoliophyta
Class	-	Magnolipsida
Order	-	Piparales
Family	-	Piperaceae
<b>Genus</b>	-	<b><i>Piper</i></b>
<b>Species</b>	-	<b><i>longum</i></b>



**Photograph of *Piper longum* plant with fruits and Root**

Long pepper is a native of Indo-Malaya region. It is found growing wild in the tropical rain forests of India, Nepal, Indonesia, Malaysia, Sri Lanka, Rio, Timor and the Philippines. Indian long pepper is mostly derived from the wild plants. The roots and fruits of the plant are used as an antidote to snake bite, scorpion stings, chronic bronchitis, cough and cold. The ripe fruits are used as an alternative to tonic. The major chemical constituents present in piper species are Alkaloids, amides, Lignans, esters and Volatile oils. The fruit contains a large number of alkaloids and related compounds, the most abundant of which is Piperine, together with methyl piperine, piperonaline, piperettine, piper longumine and Refractamide-A. The lignans are Sesamin, Pulviatol and fargesin have been isolated from fruits. The essential oil of the fruit is a complex mixture; the three major constituents are Caryophyllene, Pentadecene and bisabolone.

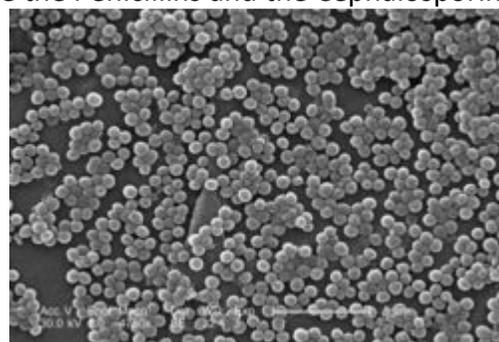


**Structure of Piperine**

The crude extract and its hexane fraction of *P. longum* exhibited antifertility effect in female rats. The fruits of *Piper longum* have been used in traditional remedies against intestinal distress. The ethanolic, hexane and n- butanol fraction exerted invitro amoebicidal activity. *Piper longum* has shown caecal amoebiasis in rats (Achenbach et al., 1986). Hence it is widely used in cooking and traditional medicine. However, as the fruits are reported to have contraceptive activity in



experimental modules, its use during pregnancy and lactation should be avoided (Mathur et al., 2004). It also possess immunomodulatory activity (Tripathi et al., 1999), Hepatoprotective activity (koul et al., 1993), Hypocholesterolemic, Antidiabetic, Antioxidant and Antilipidperoxidative activity (Agbor et al., 2007), Antiasthmatic activity and Antiinflammatory activity (Majeed et al., 2000), and **Antibacterial activity against MRSA (Inshad et al., 2005)**. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for difficult-to-treat infections in humans. It may also be referred to as multiple-resistant *Staphylococcus aureus* or oxacillin-resistant *Staphylococcus aureus* (ORSA). MRSA are by definition strains of *Staphylococcus aureus* that are resistant to a large group of antibiotics called the beta-lactams, which include the Penicillins and the Cephalosporins.



**Electron micrograph of MRSA**

*S. aureus* most commonly colonizes the respiratory tract, opened wounds, intravenous catheters, and urinary tract are also potential sites for infection. Healthy individuals may carry MRSA asymptotically for periods ranging from a few weeks to many years. Patients with compromised

immune systems are at a significantly greater risk of symptomatic secondary infection.

Ciprofloxacin, the most frequently used fluoroquinolone, is less effective against gram-positive bacteria, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococcus faecalis*. Multidrug efflux transporters also play a major role in contributing to the resistance of gram-positive organisms to fluoroquinolones by actively extruding fluoroquinolones and multiple other drugs from cells. Piperine, the major plant alkaloid present in black pepper (*Piper nigrum*) and long pepper (*Piper longum*), is reported to have bioavailability-enhancing activity for some nutritional substances and for some drugs (Ali Khan et al., 2005).

### AIM OF THE STUDY

Herbal drugs are considered to be less toxic and freer from side-effects compared to synthetic drugs. Plants have always been an exemplary source of drugs and many of the currently available drugs were derived directly or indirectly from them. Hence, in the present study, an attempt has been made to evaluate the synergistic antibacterial activity of piper longum extract with Ciprofloxacin on Methicillin-resistant *Staphylococcus aureus* (MRSA)

### MATERIALS AND METHODS

#### Collection of *costus igneus*

Fresh pepper, *Piper longum* fruits and roots were collected from Kanchipuram district, were washed thoroughly in tap water and with distilled water; shade dried for five days and grounded into uniform powder using milling machine. The powder was used for extraction preparation.

#### Chemicals

Diethyl ether, Ethanol, Methanol, Chloroform, was the major chemicals used for the present study. They were obtained from the standard chemical companies like Qualigens, SRL and CDH etc.

#### Microorganisms

Among the variety of microorganisms that are available, *Staphylococcus aureus*, was taken for the assay of antibacterial activity. These bacteria Available online on [www.ijprd.com](http://www.ijprd.com)

were obtained from the Microbiology laboratory, Sri Sankara Arts and Science College, Enathur.

#### Aqueous:-

Aqueous extract of the sample was prepared by soaking 10g of dried powder in 200ml of distilled water for 12hrs. The extract was filtered using the Whatmann filter paper no.42 (125nm) (Edeoga et al., 2005).

#### Methanol, Ethanol and Chloroform extract

5 gram of the air dried, powdered sample is packed between folds of filter paper and placed in soxhlet apparatus, run between 60 - 80°C using various solvents such as Methanol, Ethanol and Chloroform to extract the active components. The extract obtained by Soxhlet extraction procedure is used for qualitative phytochemical screening.

#### Qualitative analysis of Alkaloids in *Piper longum* – Phytochemical analysis

##### I. Phytochemical Screening Procedure:

These tests were carried out using standard procedures of Harborne (1973).

##### i) Test for Alkaloids

###### a) Mayers test:-

**Reagent:** - 1.358g of mercuric chloride was dissolved in 60ml of water. 5g of potassium iodide was dissolved in 10ml of water. The two solutions were mixed and water was added and made up to 100ml.

**Procedure:** - To a few ml of filtrate, a few drops of Mayer's reagent was added by the side of tube. A creamy white precipitate indicates the presence of alkaloids.

###### b) Wagners test:

**Reagent :-** 2g of iodine and 6g of potassium iodide we dissolved in 100ml of water.

**Procedure:** - To 2ml of filtrate, 1% HCl and 6 drops of Wagner's reagent were added. A brownish red precipitate indicates the presence of alkaloids.

###### c) Dragendorff test:-

**Reagent:** - 0.85g of basic bismuth nitrate was dissolved in 10ml acetic acid and 40ml of water. 8g of potassium iodide was dissolved in 20ml of water. 5ml of both the solutions were mixed with 20ml of acetic acid and 100ml of water before use.

**Procedure:-** To 2ml of filtrate, 1% HCl and 6 drops of Dragendorff reagent were added.

**Quantitative determination of alkaloids (Harborne et al., 1984)**

The amount of alkaloids was determined by the standard method devised by Harborne et al., 1984. To 5g of the sample in 250ml beaker, 250 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtrate. The residue is the alkaloid, which was dried and weighed. The values were expressed as mg%

**Preparation of crude extracts for Antibacterial activity**

To study the effect of various solvent extracts of piper longum for antibacterial activity, each 1gram of powder was mixed with each 10ml of different solvents such as, Diethyl ether, Ethanol, Methanol and Chloroform in a 50 ml beaker. After 12 hours, all the extracts were filtered using What mann filter paper (125mm) to remove plant debris. All the solvent extracts are concentrated by evaporation and used for testing antibacterial activity.

**Testing for Antibacterial activity**

**Table:-1** - Phytochemical Alkaloidal screening of various solvent extracts of *Piper longum*.

Phytochemicals	Fruit				Root			
	Aqueous	Methanol	Ethanol	Chloroform	Aqueous	Methanol	Ethanol	Chloro form
<b>Alkaloids determined by</b>								
<b>1. Dragendroff's Test</b>	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
<b>2. Wagner's Test</b>	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	+ ve
<b>3. Mayer's Test</b>	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve

+ Ve → indicates the presence of Alkaloids

- Ve → indicates the Absence of Alkaloids

Antibacterial activity of *Piper longum* crude extract was tested against Methicillin-resistant *Staphylococcus aureus* (MRSA) strain by disc diffusion method. 0.25mg of crude extract was impregnated on sterile 5mm diameter filter paper disc and allowed to dry. Then the crude extract impregnated discs were placed over Muller–Hinton agar medium inoculated with Methicillin-resistant *Staphylococcus aureus* (MRSA) using sterile cotton swab. Zone of inhibition was measured after 24 hours of incubation at 37°C (Gulshan et al., 2005).

An inhibition diameter of less than or equal to 7mm was considered insignificant, 8-10 mm was considered moderate inhibition and more than 10mm was taken to be significant inhibition. All the media used in the present investigation were of analytical grade obtained from Hi-Media Laboratories Ltd.

**Composition of Muller Hinton Agar**

CONTENTS	Grams/Litre
Beef infusion form	300
Casein and Hydrolysate	17.50
Starch	1.50
Agar	17.0
Final (pH) at 25°C	7.3 + 0.2

**RESULTS AND DISCUSSION**

The results of qualitative phytochemical analysis of alkaloids present in various solvent extracts of Fruit and Root samples of *Piper longum* are given in the Table-1.

The results of quantitative determination of Alkaloids carried out in aqueous extract of *Piper longum* are given in the Table-2. From this result,

**Table-2.** Quantitative estimation of Alkaloids of Aqueous extract of Fruit and Root extract of *Piper longum*.

Phytochemical quantitated	<i>Piper longum</i> (Fruit)	<i>Piper longum</i> (Root )
	Aqueous Extract	
Alkaloids	6g%	5.7g%

Piperine is an alkaloid found naturally in plants belonging to the pyridine group of Piperaceae family, such as *Piper nigrum* and *Piper longum*. Piperine is the Trans stereoisomer of 1-piperoylpiperidine. It is also known as (E, E)-1-piperoylpiperidine and (E, E)-1-[5-(1, 3-benzodioxol-5-yl)-1-oxo-2, 4-pentdienyl] piperidine. Piperine is the alkaloid responsible for the pungency of black pepper and long pepper, along with chavicine (an isomer of piperine). It has also been used in some forms of traditional medicine and as an insecticide (Nahak et al., 2004). Majeed (1999) reported that piperine is widely used in various herbal cough syrups for its potent anti-tussive and bronchodilator properties. It is used in anti inflammatory, anti malarial, anti leukemia treatment. Recent medical studies have shown that it is helpful in increasing the absorption of certain vitamins, selenium,  $\beta$ -carotene, also increase the body's natural thermogenic activity.

In the study of evaluation of antibacterial activity of Ethanolic, Methanolic, Diethyl ether and

**Table-4:** Synergistic activity of *Piper longum* extract on MRSA

S.No	Antibiotic Sensitivity Test	Zone of Inhibition in mm
1.	Antibiotic (Ciprofloxacin)	25
2.	Diethyl ether Fruit extract	21
3.	Antibiotic (Ciprofloxacin)+ Diethyl ether Fruit extract	28
4.	Antibiotic (Ciprofloxacin)	25
5.	Diethyl ether Root extract	20
6.	Antibiotic (Ciprofloxacin)+ Diethyl ether Root extract	27

The results showed that both the fruit and Root extract of *Piper longum* along with Ciprofloxacin showed good synergistic activity (28mm of inhibition) against MRSA.

Figure - A & B Shows the value of inhibition zones under antibiotic testing of diethyl ether extract

fruit of *Piper longum* contains more amounts of alkaloids when compared to root extract.

Chloroform extracts of *Piper longum*, the Diethyl ether extract of root and fruit was found to be more efficient than the other solvent extracts tested. The results of antibacterial activity of diethyl ether extract against MRSA are given in Table-3.

**Table-3:** Antibacterial activity of Diethyl ether extract of *Piper longum* against MRSA

S.No	Diethyl ether Extract	Zone of Inhibition in mm
1.	Root Extract	20
2.	Fruit extract	21

The diethyl ether extract of *Piper longum* fruit and root have significant inhibitory effect on *S.aureus* was known to cause a variety of infections namely Diarrhoea, Pneumonia and Sepsis (Inshad et al., 2005).

The synergistic activity of *Piper longum* extracts on MRSA was given in Table-4.

against MRSA. Ciprofloxacin was taken as standard, which is a broad spectrum antibiotic and acts as therapeutic targets for number of gram positive and gram negative microorganisms.

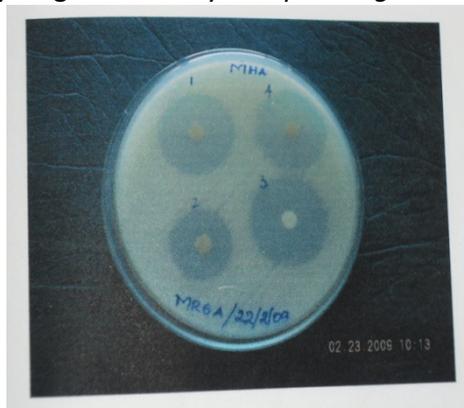
**Figure – A & B:** Synergistic activity of *Piper longum* extract with Ciprofloxacin on MRSA

Figure-A

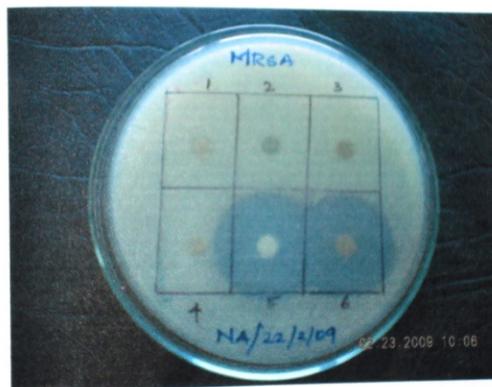


Figure-B

The diethyl ether extract is taken as control and the extract along with ciprofloxacin is taken as Test. The diameter of zone of inhibition gives a additive effect which is comparably more when compared with antibiotic or diethyl ether extract alone. Therefore the Diethyl ether extract of *Piper longum* shows significant antibacterial activity and potentiating activity with ciprofloxacin against MRSA.

The mechanism by which it can act as potentiating role as antimicrobial agent against MRSA reveals that Piperine is an P-Glycoprotein inhibitor (Multidrug efflux Transporter) that inhibits Ciprofloxacin efflux from bacterial cells (Inshad et al., 2005)

With regard to antibacterial activity of different extracts of leaves of *Piper longum*, all the crude extracts (except petroleum ether), have mild to moderate activity against most of the bacterial strains, but petroleum ether extract showed activity against only gram –positive *Streptococcus aureus* (Abbas et al., 2007)

## CONCLUSION

*Piper longum* is an alkaloidal rich plant, Piperine is the major alkaloid present in higher amount (6gm %) has effective antibacterial activity specifically against MRSA strains which cause respiratory tract diseases. So Piperine based drugs showed synergistic activity with ciprofloxacin against MRSA. Therefore Piperine derived drugs are commonly employed for treating respiratory diseases such as Tuberculosis, Asthma, and Pneumonia etc.

But before use in human being isolation of pure compound, toxicological study and clinical trial in animal model should be carried out thereafter. However, further and specific studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

## REFERENCES

1. Abbas ali m, Noos mahbub alam, Mst. Sarmina yeasmin, astaq mohal khan, M.abu sayeed, 2007. Antimicrobial screening of different extracts of piper longum. Research journal of agriculture and biological sciences. 3(6): 852-857.
2. Agbor GA, Vinson JA, Ngogang JY. Invitro antioxidant activity of three piper nigrum species 2007. Herb.j.Pharmacother, 49-64.
3. Chattejee A and Dutta C.P. Alkaloids of piper longum linn – Structure and synthesis of piper longumine. Tetrahedron (23): 1769-1781.
4. Harborne J.B., 1992. Phytochemical methods. Chapman and Hall publications, London:7-8.
5. Inshad alikhan, Zahid mehmoood, Ashwani kumar, Vijeshwar verma and Ghulam Nabi qazi, 2005. Piperine, a phytochemical potentiator of ciprofloxacin against staphylococcus aureus.
6. Kapoor L.D. Handbook of Ayurvedic medicinal plants. 264-265.
7. Koul L.B and Kapil A, 1993. Evaluation of the liver protective potential of piperine, an active principle of black and long peppers. Planta medica, 59(5):413-417.

8. Gayatri Nahak and R.K. Sahu, 2011. Phytochemical Evaluation and Antioxidant activity of Piper cubeba and Piper nigrum. Journal of Applied Pharmaceutical Science 01 (08); 2011: 153-157.

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