# Antimicrobial Activity of Piperine purified from piper nigrum

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# Antimicrobial Activity of Piperine purified from *piper nigrum*

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**Abstract:**
An alkaloid piperine was extracted from dry seeds of plant *Piper nigrum* with ethanol by using saxohlet extraction then isolation and purification by re- crystallization , the structure of Piperine was confirmed by the IR spectroscopy. Antimicrobial activity of Piperine was studied against gram+ve, gram-ve bacteria and *Candida albicans* piperine showed potent antimicrobial activity against tested organisms especially *C. albicans* followed by *E. coli* and less than on *P. aeruginosa* the zone of inhibition ranges from 8 - 23 mm and the minimum inhibitory concentrations ( MIC ) 3.125 - 100mg/ml .

## Introduction

Spices and herbs have been used for centuries by many cultures enhance the flavor and aroma of foods .Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value . Scientific experiments scince the late $19^{th}$ century have demonstrated the antimicrobial properties of some spices , herbs and their components . ( 2 , 18 ) Recently, there has been an increasing interest in the discovery of new natural antimicrobials , because of an increasing risk in the rate of infection with antibiotic- resistant microorganisms and also due to the side and the residual effects of antibiotics . The part of different common herbs , such as *Piper nigrum* , commonly known as black pepper (1) Sometimes called Indian long pepper , is a flowering rine in the family piperaceae , cultivated for its fruit , which is usually dried and used as aspice and seasoning, it is a close relative of the black pepper plant , and has a similar , through generally hotter taste . The root and fruit of *piper longum* are used in palsy , gout and lambago . The fruits have a bitter , hit , sharp taste , tonic to liver , stomatch , emmenagogue , abortifacient , aphrodisiac and digestive (11).

The fruits and roots are attributed to numerous medicinal uses and may be used for diseases of respiratory tract , cough, bronchitis, asthma, etc; as counter irritant and analgesic when applied for muscular pains and inflammation ,it is also prescribed in case of cholera, dyspepsia, flatulence and diarrhoea. (19) Several alkaloidal and non- alkaloidal constituents have been reported from time to time such as piperine and pipericine (5) .

Piperine is the major plant alkaloid present in black pepper *Piper nigrum* and long pepper *Piper longum*, is reported to have bioavailability enhancing activity for some drugs (3, 16 ).Piperine has good anticonvulsant and anti microbial properties (13) , it also acts as an antioxidant and anticancer agent by its numerous macromolecules associated with them ( 9 ) .

The purpose of the present study was to determine the antimicrobial activity of piperine that isolated from *Piper nigrum* by taking a selected standard antibiotics .

## Materials and Methods :

**Extraction and identification of the piperine .**

Fresh peppercorns ( 25 g ) were ground to affine powder , placed in asoxhlet thimble , and extracted with ethanol ( 250 ml ) for at least 90 minutes , At the end of the extraction the ethanol should be pale Yellow to colourless , the resulting solution was cooled , filtered and concentrated . The residue was dissolved in 10 % Alcoholic potassium hydroxide ( 25 ml ) . The solution was decanted if any residue remained , water drop wise was added ( about 30 ml will be required ) to precipitate the piperine. The piperine was collected and dried . Recrystallization from acetone : hexane ( 3:2 ) produced cleaner matterial . Structural determination is based on the infrared spectra ( IR spectrum ) and melting point (8) .The structure of Piperine was compared with IR standardize .

## Antimicrobial Screening

As test microorganisms , Gram ( + ) bacteria *Staphylococcus aureus* , Gram ( - ) bacteria *Escherichia coli* , *Proteus vulgaris* , *Klebsiella pneumoniae,Pseudomonas aeruginosa* and the yeast *Candida albicans* .Clinical isolates of *Proteus vulgaris* , *Klebsiella pneumoniae* were obtained from MSc.Hussein, K. Abdul-sada , while *Candida albicans* was obtained from Dr. Kawther,T. Khalaf , and *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* from MSc. Eiman, A. Saeed . pharmacology Dept. Pharmacy college .Basrah-university.

Antimicrobial activity was studied by the standard disc diffusion method (4) . Muller hinton medium was used for determining antibacterial activity whereas Potato Dextrose Agar Medium was selected for antifungal activity Standard antibiotic disc of streptomycin ( 10 µg / disc ) and erythromycin ( 15 µg / disc) were also used for comparision in antibacterial testing respectively only .The piperine 100 mg was dissolved 10 ml of methanol (90%) the control experiment was setup with 2ml of ethanol (90%) which served as control.

The antimicrobial activites were determind by measuring the diameter of the inhibitory zones in mm . The diameter of the growth inhibtion zones produced by the piperine on bacteria only were compared with diameter of the zone of the inhibition produced by the standard antibiotic disc .

Minimum inhibitory concentration (MIC)was determined by using the serial dilution method described by ( 15 ) , using the following concentration 400 , 200 , 100 , 50 , 25, 12.5 , 6.25 , 3.125 mg/ml . MIC values were determined as the lowest concentration of the constituent which completely inhibited the growth of the tested microorganisms ( 12 ) .

## Results and Discussion

piperine was obtained as purified crystals as long yellow needles with weight of 1.5 gm, fig 1, having melting point 123.
IRspectrem showed characteristic bands for C-H ( aromatic and alphatic ) ( 2800 ,3000 cm ) , N-C = 0 ( amide ) (1680 cm ) , C = C ( 1600 cm ) C-N ( 1250 cm ) C-O ( 1080 cm ) and C-H ( aromatic ) ( 1000 cm ) , the values of IR to isolated piperine in combination with standardized piperine IR fig ( 2,3 ) and these results inagreement with ( 7 ).

In the present study piperine exhibited maximum effect against *C . albicans* and *E. coli.* As the diameter of inhibitory zones were 23 , 20 mm respectively and found to be most active antibacterial agent against all bacterial isolates . Table -1 and fig 4 , 11 , the piperine has higher antimicrobial activity against *E. coli* and *P. vulgaris* in compare to erythromycin and streptomycin table - 1, fig. 4 , 5 while less activity in comparition to streptomycin and erythromycine against *S. aureus* and *P.aeruginosa* . Table -1, fig 6 ,7 , 8 , 9 and piperine showed higher antimicrobial activity against *K. pneumoniea* in compare to Erythromycin but this activity was less than Streptomycin fig.10.

The result of the minimum inhibitory concentrations is shown in table- 2 , all the studied bacteria and *C.albicans* were sensitive to piperine . MIC of piperine was between 3.125 - 100 mg / ml . The most

sensnitive microorganism was *C. albicans* with MIC 3.125. Piperine activity may be comes from its ability to interfere with DNA and protein synthesis ( 9 ) .

Piperine also act as an inhibitor of Nor A efflux pumps which ac as transport proteins involved in the release of toxic substrate from inside the cells to the external enviroment . These proteins are found in both Gram positive and negative bacteria as well as in eukaryotic organism ( 18 ) .

The higher sensitivity of *C. albicans* to piperine comes from that the piperine, inhibited the lipid accumulation in yeast. ( 6 ) ,the inhibitory effects were quantitatively represented by the total lipid accumulation amount,triacylglycerol accumulation amount ,decrease in lipid amount and decrease in lipid body size with decrease in cell growth. Inhibitory effect of these compounds lead to decrease in triacylglycerol accumulation without any additional accumulation, of its intermediates suggesting that they will suppress the total carbon inflow into the triacylglycerol biosynthesis.(10).

The sensitivity of *E.coli* to piperine may by resulting from the *E . coil* have ahighly permeable membrane in compaire to other gram negetiv bacteria , piperine alteration in the permeability of the cell wall which contain high level of lipid material (14).

**Table ( 1 ) : Antimicrobial activities of Piperine**

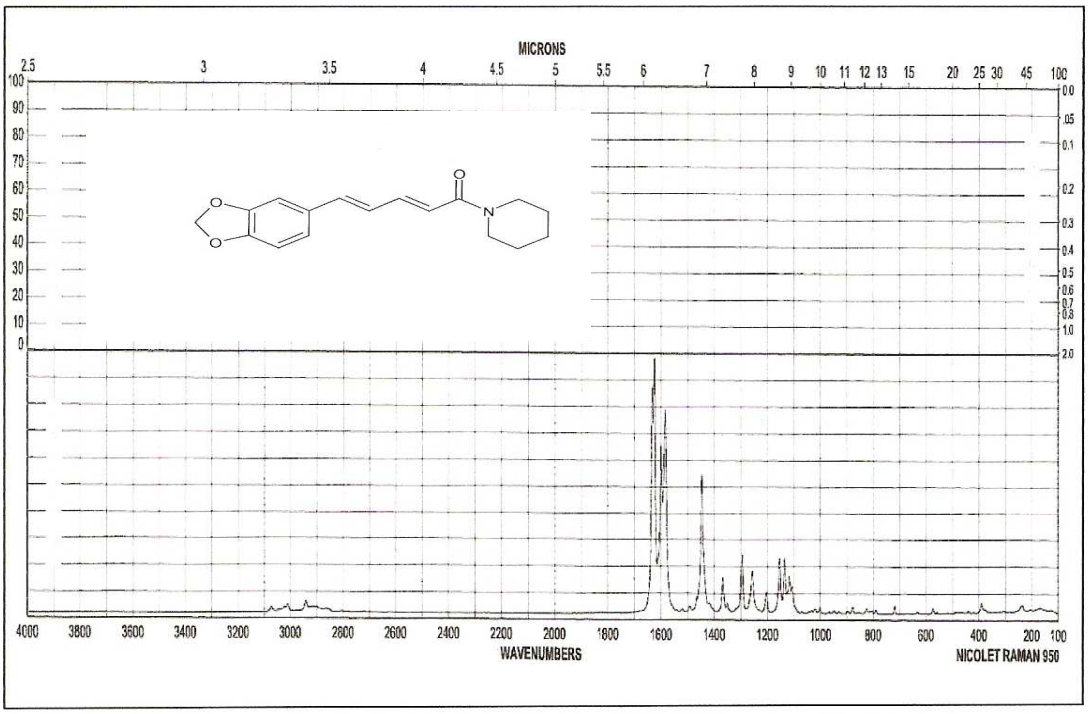
| Test organisms | piperine 100 mg / disc | Streptomycin 10 µg / disc | Erythromycin 15µ g / disc | Control Ethanol ( 90%) |
|---|---|---|---|---|
| *Escherichia coli* | 20 | 13.5 | 15 | - |
| *Staphylocuccus aureus* | 12 | 23 | 24 | - |
| *Klebsilla pneumoniae* | 15 | 20 | 10 | - |
| *Proteus vulgaris* | 17 | 10 | 6 | - |
| *Pseudomonas aeruginosa* | 8 | 22 | 18 | - |
| *Candida albicans* | 23 | No test | No test | - |

( -) = not sensitive

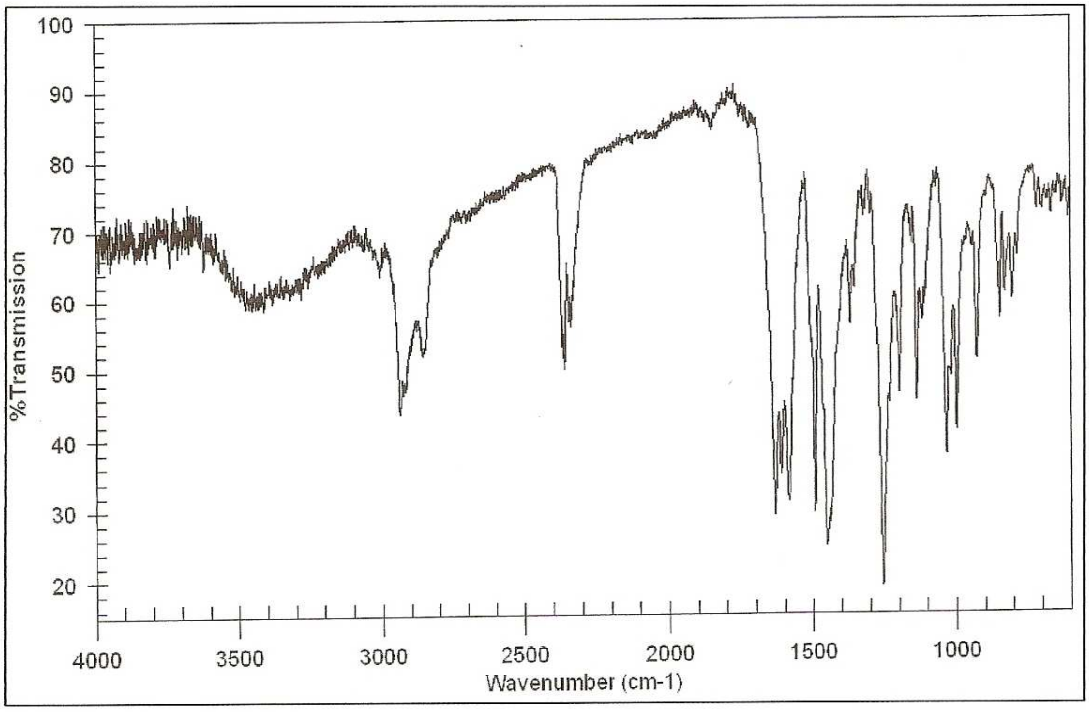
**Table( 2): MIC values of the piperine against different target organism.**

| Target organism | MIC ( mg / ml ) |
|---|---|
| *Escherichia coli* | 6.25 |
| *Staphylocuccus aureus* | 50 |
| *Proteus vulgaris* | 12.5 |
| *Pseudomonas aeruginosa* | 100 |
| *Klebsilla pneumoniae* | 25 |
| *Candida albicans* | 3.125 |

**Figure ( 1 ) : Needle crystal of piperine 16 x**




**Figure(2) : Infra-red spectra of purified piperine standard .**




**Figure(3) : Infra-red spectra of purified piperine**

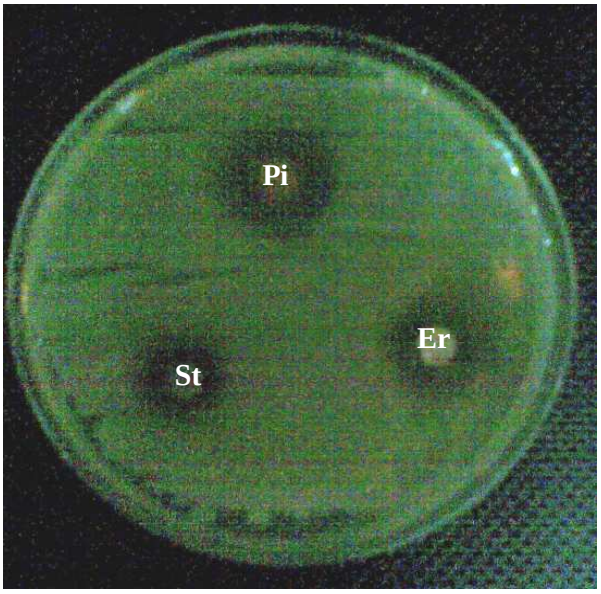



**Figure (4 ) : Activity of piperine , Erythromycine and Streptomycin on *E. coli***

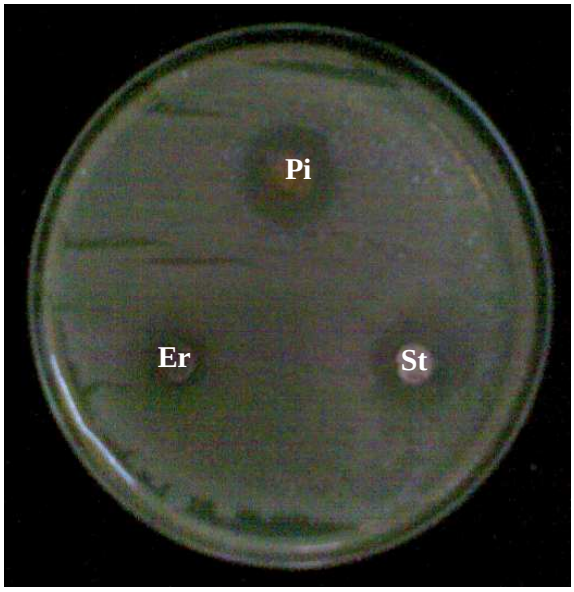



**Figure ( 5 ) : Activity of Piperine , Erythromycine and Streptomycin on *P. vulgaris***

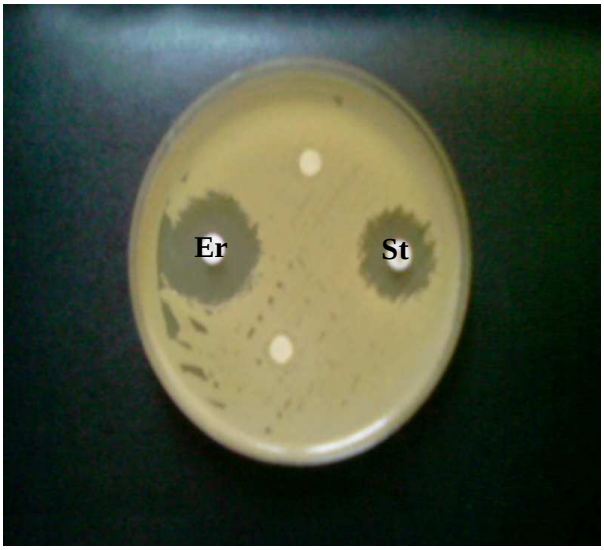



**Figure ( 6) : Activity of Erythromycine and Streptomycin on *S. aureus***

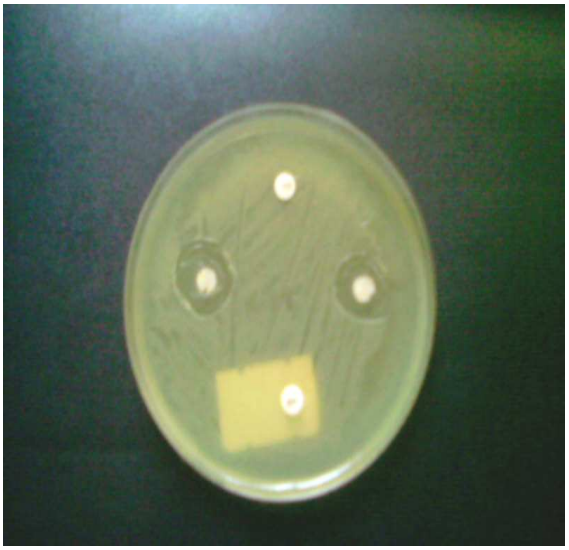


**Figure ( 7 ) : Activity of piperine on *S. aureus***

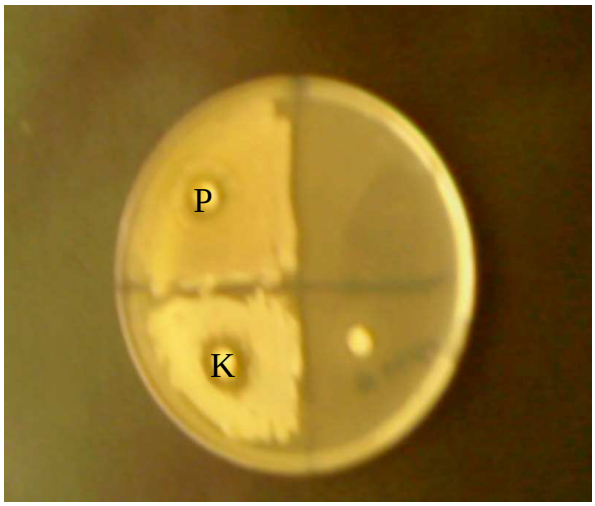



**Figure ( 8) : Activity of Erythromycine on *K. pneumonae* and *P. aeruginosa***

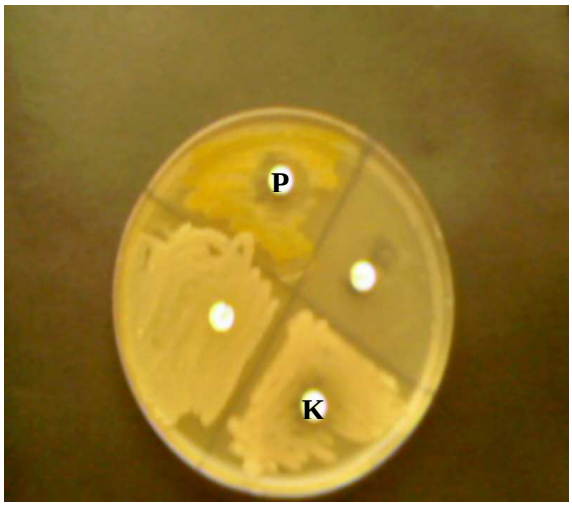



**Figure ( 9 ) : Activity of piperine on *K. pneumonae* and *P. aeruginosa***

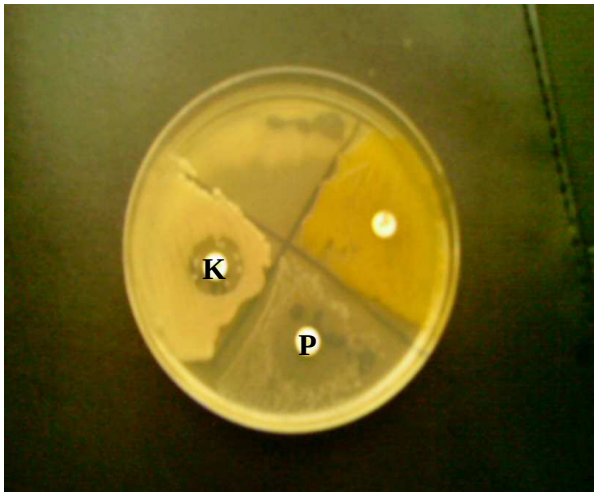



**Figure (10) : Activity of Streptomycin on *K.pneumoniae* and *P.aeruginosa***

**Figure (11 ) : Activity of piperine on *C. albicans***

## Conclusions :

Piperine aclass of compounds derived from a natural plant which is have potent activity against Gram+ve , Gram-ve bacteria and yeast *Candida albicans* and its may be signifies as a source of therapeutic agents .

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# الفعالية الضد ميكروبية للبايبرين المنقى من الفلفل الأسود *piper nigrum*

**زينـــــب طعمـــــــــة خلـــــف الدلـــــــــي**

***كلية الصيدلة ، قسم الصيدلانيات والصيدلة السريرية ، جامعة البصرة***

**المستخلـــص**

أستخلص المركب الألكلويدي piperine من البذور الجافة لنبات *Piper nigrum* بالميثانول باستخدام جهاز السكسوليت وتم عزله وتنقيته باعادة البلورة . حدد التركيب الكيميائي للبايبرين بواسطة طيف الـــ IR . درست الفعالية الـــضد ميكروبيـــة للبايبرين تجاه الجراثيم السالبة والموجبة لصبغة كرام والخمائر *Candida albicans* وقد وجد بانه ذو فعالية تثبيطية تجاه كل العزلات خصوصا ضد *C. albicans* تليها جرثومه *E. coli* واقل فعالية تجاه جرثومه *P. aeruginosa* إذ تراوحـــت أقطار التثبيط بين 8 - 23 مليمتر و التركيز المثبط الأدنى بين 100− 3.125 مليغرام / مل .