



Research Article

**ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF PIPER NIGRUM LINN  
SEED ETHANOLIC EXTRACT ON DIFFERENT PATHOGENS AND ITS  
PHYTOCHEMICAL SCREENING**

Patil Vishin Ashish\*, Nitave Sachin Annasaheb

Dr. J. J. Magdum Trust's, Anil Alias Pintu Magdum Memorial Pharmacy College, Dharangutti,  
Tal: Shirol, Dist : Kolhapur, Maharashtra.

E-mail: vishinsalunkhe@gmail.com

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**Abstract**

The ethanolic extract of *Piper nigrum, linn* (Piperaceae) was evaluated for antimicrobial activity and antifungal activity. Various concentration (5 mg/ml, 10 mg/ml and 15 mg/ml) of ethanol extract were evaluated to study the activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus flavus*, *Candida albicans*. The antibacterial activity was measured by agar well diffusion method and antifungal activity by disc diffusion method. Piperine (15 mg/ml) showed maximum zone of inhibition against Gram positive bacteria *Staphylococcus aureus* (16mm) and minimum against Gram negative bacteria *Escherichia coli* (4mm). Piperine (15mg/ml) showed maximum antifungal activity towards *Aspergillus flavus* (14mm) and *Candida albicans* (4mm). The results showed significant activity of piperine and suggesting its use as natural antimicrobial agent. Ampicillin was used as standard antibacterial drug. Normal saline solution and alcohol was used as control to study antimicrobial activity and Amphotericin B was used as antifungal drug to study antifungal activity. The results of present study indicated that ethanolic extract of *Piper nigrum, linn* shows has potent antimicrobial and antifungal activity

**Keywords:** Piperine, Antibacterial, Antifungal activity, S.aureus, E.coli, P.aeruginosa

**INTRODUCTION**

Man always been surrounded by countless microorganisms. The disease producing microbes are playing a very important role in human life. Pathogenic microorganisms are always trying to develop resistance to the various antimicrobial agents used for their control. Therefore, the chemotherapy of communicable diseases has proved to be a continuous great effort. Scientists are forever in exploring of new antimicrobial agents to run the ever increasing menace of the microbes. Thus it is of overriding importance for the microbiologists to develop new resistant strains.

Therefore, medicinal plants are gifts of nature to cure limitless number of diseases among human beings.<sup>[1]</sup>

Black pepper (*Piper nigrum* L.) is a flowering vine commonly known as 'Kalimiri' in Hindi. Black pepper is a cultivated for its fruit, which is usually dried and used as a spice and seasoning. The fruit, known as a peppercorn when dried, is approximately 5 millimeters (0.20 cm) in diameter, dark red when fully mature, and is like drupes, contains a single seed. Black pepper is native to south India, and is extensively cultivated there and elsewhere in tropical regions.<sup>[2]</sup>

Black pepper is the world's most traded spice. The spiciness of black pepper is due to the chemical piperine. In traditional medicine, black pepper has been used for digestive disorders, such as indigestion, vomiting, diarrhea, and flatulence. Suggested modern medicinal applications of black pepper

**Address for correspondence:**

Patil Vishin Ashish

E mail: vishinsalunkhe@gmail.com

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have included the treatment of cigarette withdrawal symptoms, postural instability in older adults, and swallowing difficulties in post-stroke and neurological disorder patients.<sup>[3,4]</sup>

#### MATERIAL AND METHOD

##### Collection And Authentication Of Plant

**Material:** Black pepper (*Piper nigrum*) seeds were collected from the local market of Kolhapur. After authentication, the seeds were pulverized in mechanical grinder to obtain coarse powder. The dried powder was stored in airtight bottles.

**Chemicals:** Methanol, Dextrose, Peptone, Agar, Distilled water, Ampicillin, Amphotericin B, Barium chloride dehydrate, Sulphuric acid.

**Microbial strains:** The bacterial and fungal strains for the study were obtained from Govt. Medical college, (Microbiology and bacteriology department). The fungal strains and bacterial strains used in the study are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus flavus*, *Candida albicans*.

##### Ethanolic extract of *Piper nigrum* (Black pepper)

Place 15 g of coarse black pepper powder in 250 ml round bottomed flask add 150 ml of ethanol, reflux by using Soxhlet extractor for 2 hours, filter the mixture by suction filtration and evaporate the concentrate until dry mass is obtained.

#### ASSESSMENT OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY<sup>[5,6,7]</sup>

**1. Preparation of Inoculum** – From fungal cultured slants, several colonies were transferred to 5ml of sterile distilled water. It is mixed for some seconds to ensure homogeneity and further diluted to match the turbidity with 0.5 McFarland standard solution corresponding to  $1-5 \times 10^6$  CFU/ml).

**2. Preparation of samples** – i) *piper nigrum linn* alcoholic sample solutions were prepared at 5mg/ml, 10mg/ml, 15mg/ml concentrations in alcohol ii) Amphotericin B was taken as standard antifungal drug to study antifungal activity and Ampicillin was taken as standard antibacterial drug to study antibacterial activity.

#### 3. Disc diffusion method -

- i) Sabouraud Dextrose agar is prepared as fungal media and sterilized.
- ii) All glasswares, filter disc, petriplates, extract dilutions were sterilized in autoclave.
- iii) In aseptic technique, using sterile swab a bacterial lawn is made on sterile petri plates from microbial inoculums suspension. Swab is made in one direction by rotating plate at 90°.
- iv) Sterile filter discs of 6mm diameter were impregnated with about 0.1ml/disc of each extract dilution solution and placed on agar plate in aseptic condition.
- v) Plates are incubated at 28°c-30°c for 2 days. Alcohol, Sterile distilled water are kept as control. After 2 days zone of inhibition was measured. In case of alcoholic dilutions of *piper nigrum linn* the zone of inhibition of alcohol is subtracted from control alcohol zone of inhibition.

**4. Agar Well Diffusion Method:** The antibacterial activity of piperine was evaluated by using agar well diffusion method. Bacterial cultures are mixed in nutrient agar medium and poured in Petriplates. Wells or cups of 5mm size were made with sterile borer into agar plates containing the bacterial inoculums. 2mg of crude piperine was completely dissolved in 2ml of Ethanol 95%. Antibacterial activity was measured at different concentrations of extract ranging from 5,10,15 mg/ml drugs *Piper nigrum Linn*. The zone of inhibition of alcohol is subtracted from control alcohol zone of inhibition. Ethanol 95% served as control and antibiotic Ampicillin served as standard.

#### RESULT

Piperine (15 mg/ml) showed maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (16mm) and minimum against Gram negative bacteria *Escherichia coli* (4mm). Piperine (15mg/ml) showed maximum antifungal activity towards *Aspergillus flavus* (14mm) and *Candida albicans* (4mm).

Sr. No.	Piper nigrum Linn Extract Concentration	Zone of Inhibition in mm		
		Staphylococcus aureus	Pseudomonas aeruginosa	Escherichia coli
1.	5mg/ml	6mm	10mm	4mm
2.	10mg/ml	8mm	12mm	8mm
3.	15mg/ml	12mm	16mm	14mm
4.	Ampicillin (15mg/ml)	10mm	12mm	18mm

Table 1: Effect of *Piper nigrum* Linn extract on growth of bacteria *in vitro*. Zone of inhibition (mm)

Sr. No.	Piper nigrum Linn Extract Concentration	Zone of Inhibition in mm	
		Aspergillus flavus	Candida albicans
1.	5mg/ml	4mm	6mm
2.	10mg/ml	10mm	8mm
3.	15mg/ml	14mm	4mm
4.	Amphotericin B (15mg/m)	10mm	7mm

Table 2: Effect of *Piper nigrum* Linn extract on Pathogenic fungi Zone of inhibition (mm)

CHEMICAL TESTS	RESULT
<b>Test for Saponins</b> A. Foam test	Positive
<b>Test For Steroids</b> Salkowaski test	Positive
<b>Test For Alkaloids</b> Dragendroff's test Wagner's test Mayer's test	Positive Positive Positive
<b>Test For Tannins</b> A.5% Ferric chloride B. Acetic acid test C. Dil. KMnO <sub>4</sub> Test	Positive Positive Positive
<b>Test For Flavonoids</b> A. Lead acetate test B. NaOH + Dil.acid	Positive Positive
<b>Test for Glycosides</b> Borntrager's test	Positive

Table 3: Phytochemical Evaluation of *Piper nigrum*, Linn<sup>[8]</sup>



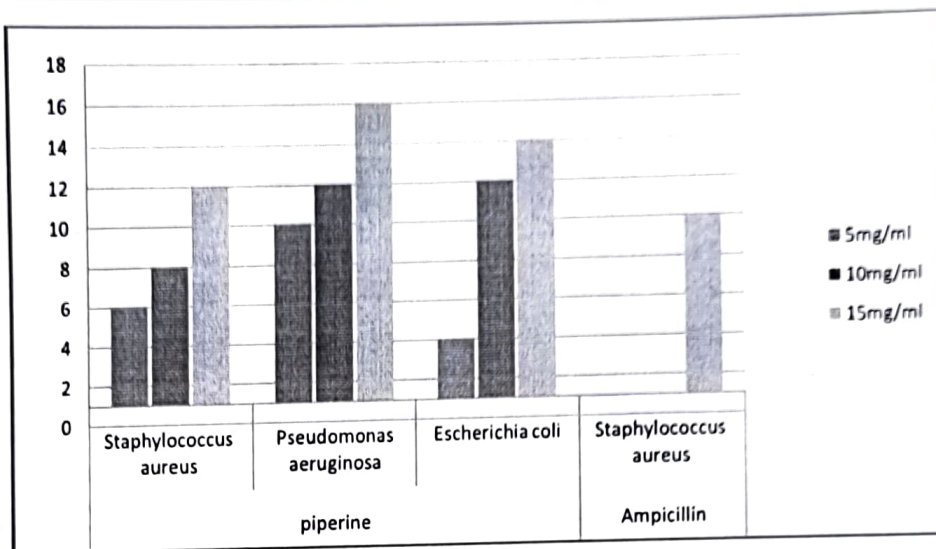


Figure 1: Effect of *Piper nigrum Linn* extract on Pathogenic bacteria Zone of inhibition (mm)  
X axis: concentration in mg/ml Y axis: zone of inhibition in mm

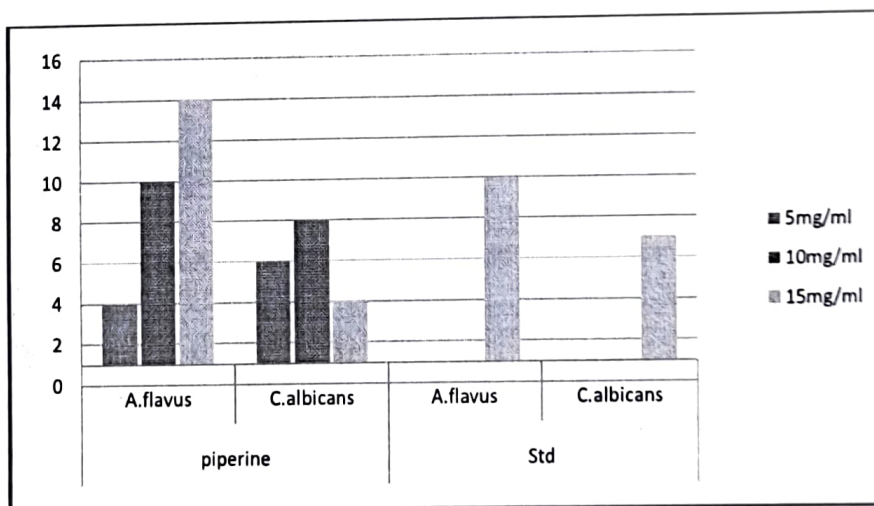


Figure 2: Effect of *Piper nigrum Linn* extract on Pathogenic fungi Zone of inhibition (mm)  
X axis: concentration in mg/ml Y axis: zone of inhibition in mm



Figure 3: Effect of alcoholic extract of *Piper nigrum linn* on *Candida albicans*.

#### DISCUSSION

The presence of antibacterial and antifungal substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines of it can be the base for the development of a medicine, a natural blueprint for the development of a drug. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The results showed significant activity of piperine and suggesting its use as natural antimicrobial agent. The result of present study indicated that ethanolic extract of *Piper nigrum, linn* shows potent antimicrobial and antifungal activity.

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#### REFERENCES

1. Bushra Beegum NR and Ganga Devi T: Antibacterial activity of selected sea weeds from Kovalam South West coast of India. Asian Journal of Microbiology Biotech Env Sci 2003; (3): 319-322.

2. Alpa Gopal Rabadia, Sheela D. Kamat, Dilip V. Kamat, Antifungal Activity of Essential Oils against Fluconazole Resistant Fungi, International Journal of Phytomedicine 3 2011, 506-510.
3. Mrs. Vishin Ashish Patil\*, Mr. Sachin Annasaheb Nitave, Comparative Anthelmintic Activity and Phytochemical Evaluation of *Tridax procumbens Linn* Whole Plant Extract and *Piper nigrum Linn* Seed Extract on Indian Adult Earthworm, I nt. J. Pharm. Sci. Rev. Res., 27(2),– August 2014; 07, 54-57.
4. Uraih, 2004. *Food Microbiology*. Bobpeco Publishers, Benin City, Nigeria, 92-130.
5. <http://e.m.wikipedia.org/wiki/McFarland-standards>
6. <http://e.m.wikipedia.org/Agar-diffusion-test>
7. S.K. Shiva Rani, Neeti Saxena and Udaysree, Antimicrobial Activity of Black Pepper, *Piper nigrum L.*, Global Journal of Pharmacology 7 (1): 87-90, 2013.
8. Manisha N Trivedi, Archana Khemani, Urmila D Vachhani, Charmi P Shah, D D Santani, Pharmacognostic, phytochemical analysis and antimicrobial activity of Two piper species, International Journal Of Comprehensive Pharmacy, 2011, 2(7), 1-4.

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