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ANTIMICROBIAL ACTIVITY OF BLACK PEPPER (*PIPER NIGRUM L.*)

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ABSTRACT

Black pepper (*Piper nigrum* L.) is a native of south India which is popularly known as "king of spices". Pepper is mostly used in the curry recipes as masalas and also as ingredient in the prescriptions of folk medicine, Ayurveda and traditional medicinal systems. The spicy tang of pepper is due to the presence of piperamides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit. Among them piperine is the major chemical constituent responsible for the bitter taste of the black pepper. In the present study piperine was evaluated for its antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis*, *Pseudomonas aeruginosa, Escherichia coli, Alternaria alternata, Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporum.* The antibacterial activity was measured by agar well diffusion method and antifungal activity by poisoned food technique. Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18mm. maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (18mm) and minimum against Gram negative bacteria *Escherichia coli* (8mm). Piperine showed maximum antifungal activity towards *Fusarium oxysporum* (14mm) and very least effect against *Aspergillus niger* (38mm). The results showed significant activity of piperine and suggesting its use as natural antimicrobial agent.

KEYWORDS: Black pepper, piperine, antibacterial and antifungal activity

INTRODUCTION

The term spices refer to aromatic or pungent vegetable substances used for flavouring foods and have several commercial uses according to (ISO). Since ancient times people used spices for preventing food deterioration and pathogenic diseases. Spices have become today as an integral part of our daily diet and many of the spices are widely used to flavour food and beverages, for food preservations, medicinal preparations, cosmetics, perfumery, bakery goods and various other products. Even today spices are used asan ingredient in drug preparations in Unani, Homeopathy and Ayurveda systems of medicine. Phytochemical investigations of the aerial parts of the plants have tartaric acid, acetic acid, citric acid, succinic acid, gums, pectin, sugars, tannins, alkaloids, flavonoids, glycosides and sesquiterpenes [1,2,7,13,]. Although, the primary purpose of spices is to impart flavour and piquancy to food, the medicinal, antimicrobial and antioxidant properties of spices have also been exploited [9]. The antimicrobial activity of is documented an alarming interest continues to the present [20].

Black pepper (*Piper nigrum* L.) is a flowering vine of the Piperaceae family that is cultivated for its fruit, which is usually dried and used as a spice and seasoning. In dried form the fruit is referred to as peppercorns. It is a native of south India and popularly known as "King of Spices". Pepper is most commonly used in curry recipes, as masalas and also included in the prescriptions of Ayurvedic and other traditional medicinal systems. Pepper is also used in folk medicine as aphrodisiac, carminative, stomachic, antiseptic diuretic and for the treatment of cough, rheumatoid arthritis, peripheral neuropathy, melanoderma and leprosy due to the presence of volatile compounds, tannins, phenols and other unknown substances [2,3,6,15].

The spicy tang of pepper is due to the presence of piperamides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit. According to [14] alkaloids play a significant role in plant physiology, agriculture, host-plant resistance, entomology, the diet and medicine. Among them piperine is the major chemical constituent responsible for the bitter taste of the black pepper. It has been found that *P. nigrum* leaf extract inhibits the growth of *Pseudomonas aeruginosa* [11,10] describes the antimicrobial activity of volatile oils of black pepper against *Bacillus subtilis*, *Pseudomonas aeruginosa, Aspergillus niger, Candida albicans* and *Saccharomyces cervisisae.* Modern day synthetic and chemical preservatives often show some negative effects or side effects, consumers tend to use naturally occurring antimicrobial components for food preservations. The main objective of this study was to evaluate the antimicrobial activity of piperine against bacteria and fungi.

MATERIAL AND METHOD

Plant Material: Black pepper *(Piper nigrum* L.) seeds were collected from the tribal villages (Paderu and chintapally) of Vishakhapatnam district of Andhra Pradesh.

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Bacterial Cultures: Gram positive bacteria Staphylococcus *aureus* and *Bacillus subtilis*, Gram negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli.*

Fungal Cultures: *Alternaria alternata, Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporum*.

Chemicals: Ethanol an organic solvent used for the extraction of alkaloid (piperine) Nutrient agar medium, Potato dextrose medium.

Extraction Procedure: 15gms of black pepper was ground finely and made into a packet. The packet was inserted into the soxhlet apparatus which was fixed it to the round bottom flask containing 300ml of ethanol and refluxed for 3hrs. Ethanol was distilled; 30ml of warm ethanolic KOH solution was added to the extract. The warm mixture was stirred and filtered to remove any insoluble matter, again the solution was warmed on a steam bath and 15-20ml of tap water was added. At this stage turbidity appeared and yellow needles (crude piperine) were settled at the bottom of the flask. Crude piperine was filtered by using Whatmann No.1 filter paper.

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 Di Methyl Sulfoxide (DMSO). Antibacterial activity was measured at different concentrations of extract ranging from 25, 50, 100 and 150ul was poured into a well of inoculated plates. DSMO served as control and antibiotic Ampicillin served as standard.

Poisoned Food Technique: The antifungal activity of piperine was evaluated by using Poisoned food technique at three concentrations (100, 500 and 1000ug/ml). Four test fungi viz., *Alternaria alternata, Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporum* were multiplied on potato dextrose agar (PDA) medium. Petriplates containing PDA supplemented with piperine extract at three concentrations with three replications were inoculated with 7-day-old culture of test fungi (5mm dia disc) and kept up-side down under aseptic conditions. PDA plate without extract is served as control and PDA plate with bavistin served as standard. The inoculated plates were incubated at 25^{0}C and colony diameter was measured and recorded after 7days. Data were statistically analyzed.

RESULTS

In the present study the antibacterial effect of piperine is showed in Table 1. Piperine showed antibacterial activity against all test bacteria with zone of inhibition ranged from 8mm-18mm. The maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (18mm) and *Bacillus subtilis* (14mm) than Gram negative bacteria *Pseudomonas aeruginosa* (9mm) and *Escherichia coli* (8mm). Maximum zone of inhibition was at 100ul for all the bacterial cultures. It indicates

that zone of inhibition increases as the concentration of piperine increased. The antifungal activity of piperine is predicted in Table 2. Piperine showed maximum antifungal activity towards *Fusarium oxysporum* (14mm), Alternaria *alternata* (17mm), minimum effect against *Aspergillus flavus* (30mm) and very least effect against *Aspergillus niger* (38mm).

Table1: Effect of piperine extract on growth of bacteria in vitro.

Sr. No.	Name of the bacteria	Zone of inhibition (mm)			
		25ul	50ul	75ul	100ul
1	*Staphylococcus aureus*	4	6	8	18
2	*Bacillus subtilis*	6	8	9	14
3	*Pseudomonas aeruginosa*	2	3	5	9
4	*Escherichia coli.*	-	5	6	8

Table 2: Effect of piperine extract on growth of pathogenic fungi in vitro

Sr. No.	Name of the pathogenic fungi	Concentration of Piperine (ug/ml)			Control
		100	500	1000	
1	*Alternaria alternata*	33	24	17	54
2	*Aspergillus niger,*	47	40	38	59
3	*Aspergillus flavus*	40	38	30	62
4	*Fusarium oxysporum*	28	20	14	54

Values are mean inhibition zone (mm) of triplicates

DISCUSSION

Antimicrobial activity of piperine increases as the concentration increases against both for bacteria and fungi. It also supports the earlier investigations[4,21]. In the present study it was revealed that Gram positive bacteria are more susceptible towards the pepper extracts than gram negative bacteria. However, there are reports of occurrence of antibacterial activity against *S. aureus, E. coli, B. megaterium, B. sphaericus, B. polymyxa* [23, 16]. Spices like turmeric and ginger extracts showed maximum activity against gram positive than compared to gram negative bacteria through individually and by mixtures [12] . The variation in the inhibition among the gram positive and gram negative bacteria is due to the cell wall and cell membrane compositions. According to[5] reported that the antifungal substances present in the plant extracts were fungistatic at lower concentrations, while become fungicidal at higher concentrations. Rhizome extract of *Curcuma longa* showed toxicity towards *Aspergillus flavus* and *Trichoderma viride* at higher concentrations. [22]. Ethanolic extraction of spices dissolves the organic compounds results in the liberation of the antimicrobial components. It is also supporting the view of [5]. Turmeric ethanolic extract showed inhibitory activity against *Rhizopus stolonifer* and *Mucor spp* [16]. Fungicidal effect of spices may be due to the lysis of fungal cell wall and cytoplasmic membrane due the liberation of antimicrobial products and it was also reported that plant lytic enzymes act on the fungal cell wall causing breakage of B-1,3 glycan, B-1,6, glycan and chitin polymer [19].Identification of medicinal plant species which possess antimicrobial activity against plant pathogens may be useful in characterization of inhibitory fractions, their synthesis and development for management of plant diseases[17] . In the present study piperine an alkaloid the major constituent of piperamides present in the skin and seed of the black pepper is responsible for the antimicrobial activity. Spices we used in our daily diet can provide protection towards bacteria and fungi. We conclude that the extracts of black pepper can be used as antimicrobial agents.

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