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Antibacterial and Antifungal Activities of easily grown Eritrean Black Pepper

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Absract: **Black pepper (piper nigrum L.) 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 system. 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 aeruginose, 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 techniques. Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18mm and maximum zone of inhibition was against Gram positive bacteria Staphylococcus aureus (18mm) and minimum against Gram negative 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.**

Key words: Black pepper- piperine-Antibacterial-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 use spices for preventing food deterioration and pathogenic disease. 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 as an 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, peetin, sugars, tannins,

alkaloids, flavonoids, glycosides and sesquiterpenes. 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. The antimicrobial activity of is documented an alarming interest continues to the present.

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. In the world it's 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. 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 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 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.

MATERIALS AND METHODS

Plant material: Black pepper (Piper nigrum L.) seeds were collected from the Eritrean supermarket. 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 used for the extraction of alkaloid (piperine) Nutrient agar medium, Potato dextrose medium.

Extraction procedure: 15gms of black pepper was ground finely and kept inside a packet. The packet was inserted into the soxhlet apparatus which was fixed in to the round bottom flask containing 300ml of ethanol and refluxed 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

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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. 2gm 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 and150ul 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 different concentrations (100, 500 and 1000µg/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 different 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 25^0C and colony diameter was measured and recorded after 7 days. 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 bacterial 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 Table2. Piperine showed maximum antifungal activity towards Fusarium oxysporum (14mm), Alternaria alternate (17mm), minimum effect against Aspergillus flavus (30mm) and very least against Aspergillus niger (38mm).

Table 1: Effect of piperine extract on growth of bacteria in vitro.

Zone of inhibition (mm)

s. n	Name of the bacteria	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 Colony diameter (mm) of pathogenic fungi

Concentration of Piperine (ug/ml)

N.o	Name of the pathogenic fungi	100	500	1000	control
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

Data given are mean of triplicates

DISCUSSION

Antimicriobial activity of piperine increases as the concentration increases against both for bacteria and fungi. It also supports the earlier investigations. In the present study it was revealed that Gram positive bacteria are more susceptible towards the pepper extracts than gram negative bacteria. The variation in the inhibition among the gram positive and gram negative bacteria is due to the cell wall and cell membrane compositions. Ethanolic extraction of spices dissolves the organic components results in the liberation of the antimicrobial components. 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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