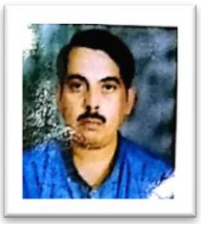
P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-3* ISSUE-3* June- 2018
E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Antibacterial activity of Indian spice Black pepper (Piper nigrum L.)



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Abstract

Many spices—such as clove, oregano, thyme, cinnamon, and cumin—possessed significant antibacterial and antifungal activities against food spoilage bacteria and even antibiotic resistant microorganisms. Piper species have been used in traditional medicine for intermittent fevers and to promote the secretion of bile. The antibacterial activity of seed extracts of Black pepper was evaluated in vitro, using well diffusion method. Three different extracts of pepper were used which included acetone, methanol and benzene. The maximum zone of inhibition was against *Pseudomonas aeruginosa* (18mm) and *Staphylococcus aureus* (18mm) at 100 µl/ml of benzene extract. Minimum activity was shown in both acetone and methanol extract. Antimicrobial activity of extracts increases as the concentration increases against all the bacteria.

Keywords: Black Pepper, Minimum Inhibitory Concentration, Pseudomonas aeruginosa and Zone of Inhibition.

Introduction

The systematic investigation of drugs of plant origin used in indigenous medicine on modern scientific lines was started more than thirty years ago and much has been accomplished during this short time. A number of important medicinal plants prescribed by the Vaids and Hakims have been carefully investigated from every point of view. Their chemical composition has been determined; the pharmacological action of the active principles worked out by animal experimentation and it is only be such a thorough enquiry that the real merits of these drugs have been proved (Zafar, 1994). Due to alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents (Ahmad et al., 1998; Bhawani and Ballow, 2000). However, there has also been a rising interest for natural products from plants for the discovery of new antimicrobial and antioxidant agents in the last three decades and in recent times. More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (Meghwal and Goswami,2012). Black pepper (*Piper nigrum*) is well-known cooking herbs and the extracts are used as medicinal products. Black pepper is produced from the still-green, unripe drupes of the pepper plant which has a bitter, hot, sharp taste, tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive. Black pepper alone accounts for about 35% of the world's total spice trade. The Materia Medica of Ayurveda, which dates back to 6,000 B.C., has many references advocating the use of pepper in a variety of ailments, particularly those pertaining to the gastro-intestinal tract (Akamasu,1970; Charaka,1941;Charlandy et al., 1999; Chopra and Chopra,1959; Kaviraj,1963; Perry,1980;Raj and Nagarsheth,1978). Piperine is the active principle alkaloid of black pepper (*Piper nigrum* L.). The piperine content is 3-9% (on dry weight basis) in *P.*. Black pepper is known to inhibit the growth of various microbes such as *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Alternaria alternata, Aspergillus Niger, Aspergillus flavus and Fusarium oxysporum* (Kaur et al., 2017).

Aim of the Study

The present investigations were undertaken to test the antimicrobial activity of seed extract of black pepper against selected pathogens.

Materials and Methods

Plant Material

The seeds of spice Black pepper (Piper nigrum L.) used for the present study were collected from the local market.

Bacterial Cultures

The microorganisms used were *Staphylococcus aureus* (NCIM-2079), *Enterobacter aerogenes* (NCIM-2695), *Escherichia coli* (NCIM-2064), and *Pseudomonas aeruginosa* (NCIM-5210).

Solvents and Media

Acetone, Benzene and Methanol solvents for extraction, Nutrient Agar.

Preparation of extract

10 gms of Black pepper was ground finely and made in to an extraction packet. The packet was inserted in to the Soxhlet apparatus which fixed in to the round bottom flask containing 100 ml of solvent. The warm mixture was stirred and filtered after distillation for 3 hours. After the complete process, the collected extracts were subjected for evaporation at room temperature. The dried extracts were stored at 4˚C for future analysis.

Agar Well Diffusion Assay

The agar well diffusion assay was employed with modifications as described by Irshad et al., 2012. Initially, autoclaved nutrient media were poured in the Petri plates under laminar air flow and after solidification of media the bacterial suspension (24 hrs old) swab over the media. The wells were prepared using cork borer. Test sample was dissolved in DMSO in different concentrations such as 25, 50, 100 µg/ml and 40 µl dissolved test sample from each concentration was loaded to the wells and incubated for 24 hrs at 37ºC. DMSO (Di Methyl Sulfoxide) used as a negative control whereas antibiotic amoxicillin disc having amoxicillin 10µg concentration used as positive control.

Results and Discussion

In present study antimicrobial activity of Black pepper was evaluated. Table-1 shown the antimicrobial activity of spice extracted in acetone, methanol and benzene against *Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli* and *Pseudomonas aeruginosa*. The maximum zone of inhibition was against *Pseudomonas aeruginosa* (18mm) and *Staphylococcus aureus* (18mm) at 100 µl/ml of benzene extract followed by 50 µl/ml benzene extract (16mm) against both the bacteria. 100 µl/ml acetone extract showed 15mm zone of inhibition followed by 50 µl/ml against *Staphylococcus aureus.*100 µl/ml methanol extract showed 14 mm zone of inhibition against *Pseudomonas aeruginosa.* The results suggest that black pepper produced significant antimicrobial effects. In this study antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was maximum shown in benzene extract of black pepper extract. Minimum activity was shown in both acetone and methanol extract. Antimicrobial activity of extracts increases as the concentration increases against all the bacteria. *E. coli* and *Enterobacter aerogenes* was least affected by all three extracts. The data supports the hypothesis that black pepper has an inhibitory effect on the growth of certain pathogens.

Conclusion

The results suggest that black pepper produced significant antimicrobial effects. In this sense black pepper can benefits of modern science and technology to serves further global needs, and may have the possibility of using in medicine.

Table 1: Effect of Black pepper extract on growth of bacteria *in vitro*

Bacteria	Dilution of Plant Extracts(µl/ml)									Negative Control	Amoxycillin (Positive control)
	Benzene			Methanol			Acetone				
	25	50	100	25	50	100	25	50	100		
	Zone of Inhibition										
Staphylococcus aureus	6	16	18	-	-	8	5	14	15	-	21
Pseudomonas aeruginosa	7	16	18	4	8	14	-	-	7	-	22
Escherichia coli	-	5	7	-	-	6	-	-	6	-	6
Enterobacter aerogenes	-	11	12	-	-	8	-	-	7	-	22

Figure 1: Inhibition Zone Photographs of *Pseudomonas Aeruginosa* for Benzene Extract of Black Pepper.

Figure 2: Inhibition Zone Photographs of *Staphylococcus aureus* for benzene extract of Black Pepper.

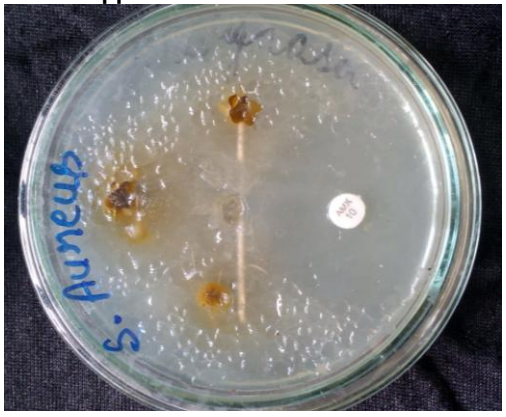
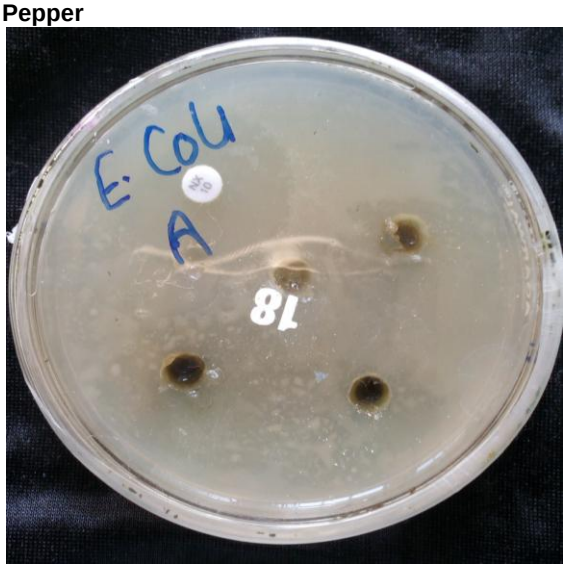


Figure 3: Inhibition Zone Photographs of *Escherichia coli* for Benzene Extract of Black Pepper



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