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Antibacterial activity of black pepper (*Piper nigrum* Linn.) with special reference to its mode of action on bacteria

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During present study the antibacterial activity of black pepper (*Piper nigrum* Linn.) and its mode of action on bacteria were done. The extracts of black pepper were evaluated for antibacterial activity by disc diffusion method. The minimum inhibitory concentration (MIC) was determined by tube dilution method and mode of action was studied on membrane leakage of UV_{260} and UV_{280} absorbing material spectrophotometrically.

The diameter of the zone of inhibition against various Gram positive and Gram negative bacteria was measured. The MIC was found to be 50-500ppm. Black pepper altered the membrane permeability resulting the leakage of the UV_{260} and UV_{280} absorbing material i.e., nucleic acids and proteins into the extra cellular medium. The results indicate excellent inhibition on the growth of Gram positive bacteria like *Staphylococcus aureus*, followed by *Bacillus cereus* and *Streptococcus faecalis*. Among the Gram negative bacteria *Pseudomonas aeruginosa* was more susceptible followed by *Salmonella typhi* and *Escherichia coli.*

Keywords: Antibacterial activity, Black pepper, *Piper nigrum*, Gram positive bacteria, Gram negative bacteria, Nucleic acids.
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Introduction

The plant extracts and secondary metabolites possess antimicrobial, antifungal or antiviral activities. The various plant products, that are regularly used for their therapeutic potential, and plants or plant products that form the part of the food or as dietary components, have been receiving considerable attention. Though much is known about the chemistry and the antimicrobial action of several phytochemicals, very few reports are available on the possible mechanism of action. For phenols and phenolic compounds, an injury of membrane functions has been proposed as a mechanism of action[1-4].

Black pepper (*Piper nigrum* Linn.) is a flowering vine of Piperaceae family. It is native to India and has been a prized spice since ancient times. The volatile oil of pepper has been shown to have antimicrobial acitivity[5]. Black pepper has many medicinal properties like it is used to treat vertigo, asthma, chronic indigestion, colon toxins, obesity, sinusitis, congestion, fever, paralytic, arthritic disorders and also advised in diarrhoea and cholera[3, 6].

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In present study the antibacterial activity of black pepper and its mode of action on bacteria by membrane leakage studies i.e., leakage of UV_{260} and UV_{280} absorbing materials spectrophotometrically were done.

Materials and Methods

Plant material

Black pepper (*Piper nigrum*) berries were purchased from local market.

Bacterial cultures

Gram positive bacteria: *Staphylococcus aureus* (NCIM-2079), *Bacillus cereus* (NCIM-2016) and *Streptococcus faecalis* (NCIM-2063) and Gram negative bacteria: *Escherichia coli* (NCIM-2089), *Klebsiella pneumoniae* (NCIM -2957), *Pseudomonas aeruginosa* (*NCIM-2200*), and *Salmonella typhi* (NCIM-2263) were obtained from National Collection of Industrial Micro-organisms (NCIM), NCL, Pune.

Chemicals

Acetone and Dichloromethane (DCM) as solvents for the extraction of black pepper (Berry), nutrient agar and nutrient broth for bacterial cultivation, standard antibiotic like Ampicillin were purchased from HI Media laboratories India, Ltd and Piperine from M/s Sigma Chemicals.

The plant material was ground to a fine powder before extraction. The pepper powder (50g) was then extracted with two solvents, viz. acetone and DCM using soxhlet apparatus by continuous heat extraction for 24 hours. The extracts obtained were concentrated to dryness by evaporating the solvent under reduced pressure[7]. The concentration thus obtained was dissolved in DMSO in such a way that the final concentration of the extract would be 1g/ml of DMSO.

Disc diffusion method

The *in vitro* antibacterial activity of the acetone and DCM extracts of pepper was carried out by disc diffusion method[8]. Actively growing log phase cultures were mixed in soft agar (Nutrient broth with 1% agar) and plated. The various extracts (5µl or 5mg) were loaded onto different filter paper discs prepared from Whatman No: 1 filter paper. The discs were then placed on the agar medium containing the cultures and incubated for 24h at 37°C. The diameter of zone of growth inhibition was recorded. The effects were compared with that of the standard antibiotic Ampicillin (10µg/disc) and DMSO alone served as control.

Minimum inhibitory concentration (MIC)

MIC was determined by serial dilution method[9]. Two fold serial dilution of the test compound was carried out in the nutrient broth. To each test tube 10^5 CFU/ml of actively growing bacterial cultures in log phase was inoculated. The culture tubes were incubated at 37°C for 24 hours. After the incubation the tubes are checked for the growth of bacteria and MIC of that extract was determined and expressed in ppm.

Mode of action of pepper extract on bacteria

The method of Heipieper[10] was followed to determine the leakage of UV_{260} and UV_{280} absorbing material. The bacterial suspension was prepared. *S. aureus* cells were grown overnight with continuous shaking in nutrient broth at 37°C, harvested, washed with 10mm EDTA and then twice in distilled water by centrifugation each time at 6000 rpm for 15 min at 4°C and resuspended such that the absorbance of the final suspension was 2.0 at A_{450}. After incubation for 30 min at room temperature, the test compound was added to suspension at MIC. At regular intervals of 15 min aliquots of the samples were drawn, centrifuged and UV_{260} and UV_{280} absorbing material in the suspension was measured.

Phytochemical screening

A small portion of the dry extract was used for phytochemical screening test[11, 12].

Results and Discussion

The zone of inhibition was measured for both acetone and DCM extract of pepper and the results depicted in Table 1. It was found that Gram positive bacteria were more susceptible than Gram negative bacteria but less efficient than that of standard antibiotic, Ampicillin.

The acetone extract of black pepper displayed excellent inhibition on the growth of Gram positive bacteria. *Staphylococcus* was more susceptible followed by *Bacillus* and S*treptococcus.* The MIC values are 125, 250 and 500 ppm, respectively. Among the Gram negative bacteria *Pseudomonas* was more susceptible to black pepper followed by *E. coli*, *Klebsiella* and *Salmonella* (62.5, 125 and 250 ppm, respectively).

The DCM extract of black pepper showed good activity and inhibited both Gram positive and Gram negative bacteria. The minimum inhibitory concentration ranged between 62.5 to 125 ppm for Gram positive bacteria and 125 to 250 ppm for Gram negative bacteria (Table 2). The active principle

Table 1—Antibacterial activity of Black pepper extracts determined by disc diffusion method

Bacteria	Zone of inhibition (mm)		
	Acetone extract (5µl)	DCM extract (5µl)	Ampicillin (10 µg/disc)
Staphylococcus aureus	20	14	22
Bacillus cereus	15	12	19
Streptococcus faecalis	18	15	13
Pseudomonas aeruginosa	15	14	24
Escherichia coli	10	NI	18
Klebsiella pneumoniae	10	12	20
Salmonella typhi	14	NI	26

NI-No inhibition

Table 2—Determination of minimum inhibitory concentration of Black pepper extracts in ppm

Bacteria	MIC in ppm			
	Acetone extract	DCM extract	Piperine	Ampicillin
Staphylococcus aureus	125	125	250	0.98
Bacillus cereus	250	62.5	250	125
Streptococcus faecalis	500	125	250	1.9
Pseudomonas aeruginosa	62.5	125	250	0.98
Escherichia coli	125	125	250	1.9
Klebsiella pneumoniae	125	125	250	1.9
Salmonella typhi	250	250	250	15.625

Table 3—Determination of leakage of UV_{260} and UV_{280} absorbing material from *Staphylococcus aureus* spectrophotometrically

Time in minutes	Absorbance at	
	UV_{260} nm	UV_{280} nm
0	0	0
15	0.08	0.3
30	0.1	0.3
35	0.1	0.3
60	0.1	0.3
75	0.2	0.3
90	0.34	0.31

piperine alone showed excellent bactericidal activity at 250 ppm against all the Gram positive and Gram negative bacteria tested.

Study on leakage of UV_{260} and UV_{280} absorbing material (mainly nucleic acid material and protein) was monitored over a period of 90 minutes. The absorbance at 280nm (protein) was increased in 15min only when compared to the absorbance at 260nm (nucleic acid material), which took 75min (Table 3). The results suggest that pepper alters the membrane permeability resulting the leakage of the UV_{260} and UV_{280} absorbing material. Leakage of intracellular materials might cause cell death, which seems to be one of the modes of bactericidal action.

The phytochemical analysis showed the presence of alkaloids, volatile oil, mono- and polysaccharides and resins. The alkaloids like piperine, piperidine, volatile oil and resins might be responsible for the antibacterial activity.

Conclusion

Present study shows that both the extracts of black pepper have good antibacterial activity, but when compared to Gram negative bacteria, Gram positive bacteria are more susceptible to the extracts. The mechanism of antibacterial action appears to be loss of control over cell membrane permeability.

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