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PHYTOCHEMICAL SCREENING OF *PIPER NIGRUM* AND *MALLOTUS PHILIPINESIS* FOR ITS
ANTIBACTERIAL ACTIVITY

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Abstract: The antibacterial activity of *Piper nigrum* and *Mallotus philipinesis* were determined against a wide variety of pathogenic bacteria. Acetone & dichloromethan extracts of *Piper nigrum* showed mild to moderate activities against most of the tested bacteria. Dichlorom ethane has very potent activity than acetone. Methanol and ethyacetate extract of *Mallotus philipinesis* methanol has potent antibacterial activity. Present study shows that both the extracts of *Piper nigrum* ant mallotus philipinesis have good antibacterial activity, but when compared with bacteri, Gram positive bacteria are more susceptible to the extracts than the Gram negative bacteria. The mechanism of antibacterial action appears to be loss of control over cell membrane permeability.

Keywords: antibacterial activity, *Piper nigrum*, *Mallotus philipinesis*

Introduction

The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. *Piper nigrum* Linn., sometimes called Indian Long Pepper, is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as a spice and seasoning. It is a close relative of the black pepper plant, and has a similar, though

generally hotter, taste. The root and fruit of *Piper nigrum* are used in palsy, gout and lumbago. The fruits have a bitter, hot, sharp taste, tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive¹. They have a pungent pepper-like taste and produce salivation and numbness of the mouth. The fruits and roots are attributed with numerous medicinal uses, and may be used for diseases of respiratory tract, viz. cough, bronchitis, asthma etc; as counter-irritant and analgesic when applied locally for muscular pains and inflammation; as snuff in coma and drowsiness and internally as carminative. Besides fruits, the roots and thicker parts of stem are cut and dried and used as an important drug in the Ayurvedic and Unani systems². The antimicrobial properties of plants have been investigated by a number of researchers world wide, especially in Latin America. In Argentina, a research tested 122 known plant species used for therapeutic treatments. Mallotus is a common tree found throughout the tropical regions of India. According to reference literatures, tree is a source of Kamela dyewhich is used in colouring silk and wool. Also it is used as anti-oxidant for ghee, vegetable oils. Kamala oil is used in formation of hair fixers and oinments. Seed oil is used in paints and varnishes and seed cake as manure. Wood pulp is suitable for writing and printing-paper. In the present study acetone and dichloromethane extract of piper nigrum and methanol and ethyl acetate extract of mallotus philipinesis was tested against 5 strain of gram positive bacteria and 5 strain of gram negative bacteria.

Materials and Methods

Plant material

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 action¹⁻⁴. Mallotus philippinensis var. Tomentosus is a medicinal plant, which was tested against gram positive and gram negative bacteria. Phytochemical screening of the stem bark of M. philippinensis indicates the presence of secondary metabolites.

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 activity⁵. 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}

Micro organisms:

Bacterial strains to be used were selected on the basis of their pathogenicity. The diseases are caused by gram positive and gram negative bacterial species and so, 5 strains of gram positive and 5 strain of gram negative strain were taken for the antibacterial susceptibility tests. Bacterial strains were obtained from MTCC, Chandigarh which are summarized in Table 1. The freeze dried powers of the strains were sub cultured in their respective growth medium. The growing conditions with the growth medium of the organisms are described in table-2.

Table-1: Bacterial strains were obtained from MTCC, Chandigarh.

Sr. no	Bacterial strains	MTCC no.
	Gram positive bacteria	
1.	<i>Staphylococcus aureus</i>	2079
2.	<i>Bacillus cereus</i>	2016
3.	<i>Streptococcus faecalis</i>	2063
4.	<i>Bacillus megaterium</i>	227
5.	<i>Streptococcus â- haemolyticus</i>	183
	Gram negative bacteria	
6.	<i>Shigella dysenteriae</i>	184
7.	<i>Escherichia coli</i>	2089
8.	<i>Klebsiella pneumoniae</i>	2957
9.	<i>Pseudomonas aeruginosa</i>	2200
10.	<i>Salmonella typhi</i>	2263

Table-2: Growing conditions with the growth medium of the fungal strains.

Bacterial strains	Growth medium	Growth condition, temperature	Incubation time
<i>Staphylococcus aureus</i>	blood agar	Aerobic; 22°C	4 days
<i>Bacillus cereus</i>	MacConkey agar	Aerobic; 25°C	1 days
<i>Streptococcus faecalis</i>	blood agar extract agar	Aerobic; 37°C	5 days
<i>Bacillus megaterium</i>	Neutrient agar	Aerobic; 28°C	6 days
<i>Streptococcus â- haemolyticus</i>	Blood agar	Aerobic; 30°C	7 days
<i>Shigella dysenteriae</i>	MacConkey agar	Aerobic; 37°C	7 days
<i>Pseudomonas aeruginosa</i>	MacConkey agar	Aerobic; 42°C	6 days
<i>Escherichia coli</i>	MacConkey agar	Aerobic; 50°C	8 days
<i>Klebsiella pneumoniae</i>	MacConkey agar	Aerobic; 50°C	10 days
<i>Salmonell typhi</i>	Potato dextrose agar	Aerobic; 38°C	6 days

Chemicals

Acetone and Dichloromethane (DCM) as solvents for the extraction of pipernigrum and methanol and ethyl acetate solvent for the extraction of mallotus philipinesis is used. Different growth media was used for bacterial cultivation, standard antibiotic like Ampicillin and kanamycin were purchased from HI Media laboratories India, Ltd and Piperine and rottlerin 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 pressure⁷. 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. The kamala powder extracted with methanol and ethyl acetate using soxhlet apparatus by continuous heat extraction for 24 hours.

Disc diffusion method

The in vitro antibacterial activity of the acetone and DCM extracts of pepper and methanol and ethyl acetate extract of kamala powder were carried out by disc diffusion method⁸. 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 Kanamycin served as control.

Minimum inhibitory concentration (MIC)

MIC was determined by serial dilution method⁹. Two fold serial dilution of the test compound was carried out in the nutrient broth. To each test tube 10⁵ 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.

Results and Discussion

The zone of inhibition was measured for both acetone and DCM extract of pepper and methanol and ethyl acetate extract of kamala the results depicted in Table 3. It was found that Gram positive bacteria were more susceptible than Gram negative bacteria but less efficient than that of standard antibiotic, Ampicillin and kanamicyn. The acetone extract of Piper nigrum displayed excellent inhibition on the growth of Gram positive bacteria. Staphylococcus was more susceptible followed by Bacillus and Streptococcus. 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). *Shigella dysenteriae* does not show any activity and *Salmonell typhi* have mordant activity against

methanol and ethyl acetate.

Table-3: Antibacterial activity of piper nigrum and mallotus philipinesis extracts determined by disc diffusion method

Bacteria	Zone of inhibition (mm) piper nigrum			Zone of inhibition (mm) mallotus philipinesis		
	Acetone extract (5µl)	DCM extract (5µl)	Ampicillin (10µg/disc)	Methanol extract (5µl)	Ethyl acetate extract (5µl)	kanamycin (10µg/disc)
<i>Staphylococcus aureus</i>	21	15	24	15	17	23
<i>Bacillus cereus</i>	16	11	18	9	11	24
<i>Streptococcus faecalis</i>	17	14	15	19	14	20
<i>Bacillus megaterium</i>	15	12	17	12	10	18
<i>Streptococcus</i> $\hat{\alpha}$ - <i>haemolyticus</i>	14	13	20	18	12	23
Gram negative Bacteria						
<i>Shigella dysenteriae</i>	12	11	13	00	00	23
<i>Pseudomonas aeruginosa</i>	15	14	24	9	11	20
<i>Escherichia coli</i>	10	NI	18	7	12	20
<i>Klebsiella pneumoniae</i>	10	12	20	00	8	26
<i>Salmonell typhi</i>	14	12	22	9	7	23

The DCM extract of Piper nigrum 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 4). The Acetone extract of Piper nigrum showed excellent activity against gram positive and gram negative bacteria it show excellent activity of

Streptococcus faecalis and poor activity of *Pseudomonas aeruginosa*. The active principle piperine showed excellent bactericidal activity at 250 ppm against all the Gram positive and Gram negative bacteria tested. another hand methanol extract of mallotus philipinesis show excellent activity as acetone extract accept *Pseudomonas aeruginosa*. *Staphylococcus aureus* and *Streptococcus faecalis* have excellent activity with methanol extract. Ethyl acetate showed the minimum inhibitory concentration ranged between 62.5 to 125 ppm for both Gram positive and Gram negative bacteria. The active principle rottlerin showed excellent bactericidal activity at range 125-250 ppm against all the Gram positive and Gram negative bacteria tested. 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.

Table-4: Determination of minimum inhibitory concentration of piper nigrum and mallotus philipinesis extracts in ppm.

Bacteria	MIC in ppm				MIC in ppm			
	Acetone extract (5µl)	DCM extract (5µl)	Piperin	Ampicillin (10µg/disc)	Methanol extract (5µl)	Ethyl acetate extract (5µl)	Rottlerin	Kanamycin (10µg/disc)
<i>Staphylococcus aureus</i>	125	125	250	0.98	500	62.5	250	0.8
<i>Bacillus cereus</i>	250	62.5	250	1.8	125	125	250	1.9
<i>Streptococcus faecalis</i>	500	125	250	1.9	500	125	125	1.8
<i>Bacillus megaterium</i>	125	125	125	1.9	250	125	125	1.9
<i>Streptococcus α-haemolyticus</i>	250	125	125	0.9	125	125	125	0.8
Gram negative Bacteria								
<i>Shigella dysenteriae</i>	250	62.5	125	0.9	250	125	125	0.8
<i>Pseudomonas aeruginosa</i>	62.5	125	250	0.98	62.5	62.5	125	0.8
<i>Escherichia coli</i>	125	125	250	1.9	250	125	250	1.8
<i>Klebsiella pneumonia</i>	125	125	250	1.9	125	125	250	10.8
<i>Salmonella typhi</i>	250	250	250	15.625	250	250	250	1.8

Conclusion

Present study shows that both the extracts of Piper nigrum & mallotus philipinesis 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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