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Original Research Paper

ANTIBACTERIAL ACTIVITY OF PIPERLONGUMINE AN ALKALOID ISOLATED FROM METHANOLIC ROOT EXTRACT OF *PIPER LONGUM* L.

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ABSTRACT

Antibacterial activity of piperlongumine was evaluated against 18 clinically isolated strains, including identified strains belongs to *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, using the agar-well diffusion method. All the clinical strains showed concentration dependent susceptibility towards the constituent piperlongumine (25, 50, 100µg/100µL). It exhibited significant zone of inhibition against both the clinical and ATCC and MTCC strains. The antibacterial activity was more pronounced against *Klebsiella pneumonia* (24.00±0.12 mm) and *Pseudomonas aeruginosa* (20.00±0.12 mm) while it was moderate on *Staphylococcus aureus* (16.47±0.18 mm). The isolated constituent was found to show better activity profile, which indicates that the isolated constituent might be responsible for the antibacterial activity. The antibacterial activity was assessed comparatively with the standard drug Ciprofloxacin.

Keywords: Antibacterial activity, *Piper longum*, Piperlongumine, Spice, Alkaloid.

INTRODUCTION

Since ancient times, plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have made a large contribution to human health and well-being. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified manner.[1,2,3] Over the past few years, many efforts have been made to discover new antimicrobial compounds from various kinds of natural sources such as microorganisms, animals and plants. In this regard several

Indian medicinal plants have been evaluated, a fair number of which possess potential antimicrobial activity[4] and few natural products have been approved as new antibacterial drugs.[5,6] However, the increased prevalence of antibiotic resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control some bacterial diseases. Hence, research for identifying novel substances that are active against human pathogens is an urgent need.[7]

Plants are known to produce some chemicals that are naturally toxic to bacteria.[8] Plant-based natural constituents can be derived from any part of the plant.[9] The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct.[10]

Piper longum L. is an aromatic climber with stout roots, jointed stems, and ovate leaves belongs to the family Piperaceae, which is very sparsely distributed in forests of the Western Ghats, India.[11] In Indian system of medicine 'Ayurveda', the plant is popularly known as Pippali. The root have been used as stomachic, thermogenic, aphrodisiac, carminative, expectorant, laxative, digestive, emollient, antigiardias, antiamoebic, anti-asthmatic, antiseptic and also active against bacterial diseases.[12,13] The plant also finds folkloric usage in the treatment of constipation, cardiac diseases, piles, liver disorders, and urinary disorders.[11]

Hence, the aim of the present study was to isolate piperlongmine from roots of *Piper longum* and to study their anti bacterial activity against pathogenic clinical strains of *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococci aureus* isolated from different infectious sources.

MATERIALS AND METHODS

Plant Material

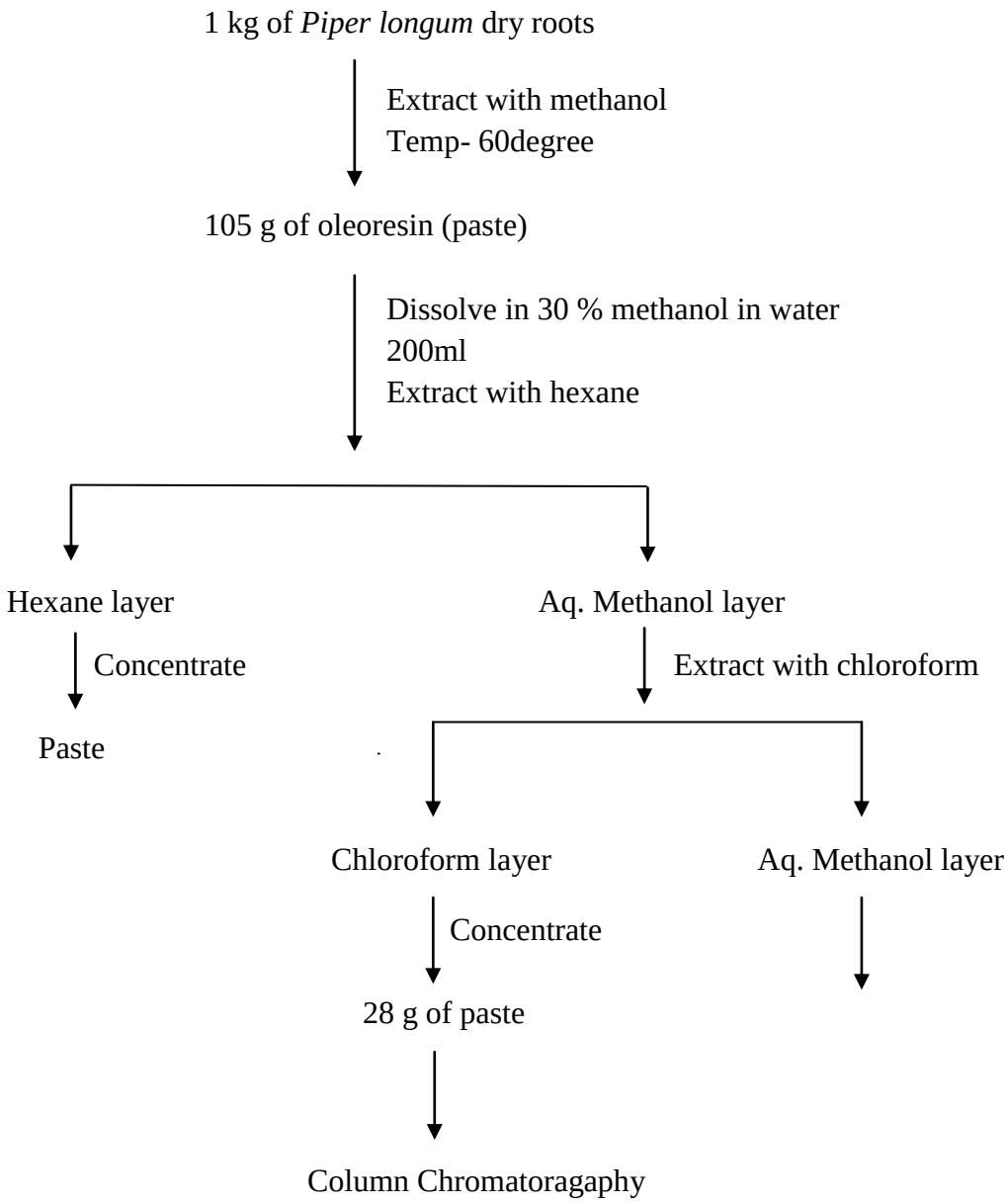
The dried roots of *Piper longum* were collected from Somavarpet region of Coorg district, Karnataka, India. The plant material was authenticated by Dr. Raja Naika, Department of Applied Botany, Kuvempu University, Shankaraghatta (Voucher specimen number AB.PL.214).

Chemicals

n- Hexane, methanol, choloroform, ethyl acetate, dimethylsulfoxide were purchased from Merck India limited, Mumbai, India. Nutrient agar was purchased from Hi-Media, Mumbai, India. Silica gel chromatographic grade for the separation was obtained from Merck, India. And other required chemicals were purchased from authorised chemical company dealers. The chemicals used in the experiments are of high purity

Extraction and Isolation

Dried roots of *Piper longum* were powdered. 1 kg dried powder was extracted with methanol in soxhlet apparatus at 60^{o}C for 48 hrs. The resultant extractive was concentrated on rota vapour (Buchi Flawil, Switzerland). The pasty mass was fractionated with hexane and chloroform. The chloroform extract was then subjected to column chromatography. Column was packed with silica gel (60-120 mesh size) in hexane and eluted with 2% ethyl acetate in hexane. 1gm of pure compound was obtained at 8% ethyl acetate in hexane and the compound was characterized with the help of NMR and Mass spectroscopy. The isolated constituent was then subjected for the evaluation of antibacterial activity.



Scheme 1: Extraction and Isolation.

Bacterial Culture

The bacterial strains used in this study were clinical isolates from different infection status of patients presenting symptoms of *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* - associated diseases. The isolates were identified by a standard method.[14] The standard strains used were *Klebsiella pneumoniae* (MTCC-618), *Pseudomonas aeruginosa* (ATCC-20852) and *Staphylococcus aureus* (ATCC-29737). The organisms were maintained on nutrient agar slope at 4^{o}C and sub-cultured into nutrient broth by a picking-off technique[15] for 24 hrs before use.

Bacterial Susceptibility Testing

In vitro antibacterial activity of piperlongumine was studied against Gram-negative and Gram-positive bacteria by the agar well diffusion method.[16] Nutrient agar (HiMedia, India) was used as the

bacteriological medium. The piperlongumine was dissolved in 10% aqueous dimethyl-sulfoxide (DMSO) to a final concentration of 25, 50, 100 µg/100µl. Pure DMSO was taken as the negative control and 50 µg/100µl Ciprofloxacin as the positive control.

100 µl of inoculum was aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculum. Wells were prepared in the agar plates using a sterile cork borer of 6.0 mm diameter. 100 µl of test and control compound was introduced in the well. The same procedure was used for all the strains. The plates were incubated aerobically at 35^{o}C and examined after 24 hours.[17, 18] The diameter of the zone of inhibition produced by different concentration of piperlongumine were measured with a ruler and compared with those produced by the commercial antibiotic Ciprofloxacin. The results of the experiment are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple pairwise comparison tests, using software ezANOVA ver. 0.98.

RESULTS

Characterization of Isolated Compound

^{1}H NMR and MASS spectral analysis confirmed the chemical structure of the compound. ^{1}H NMR spectrum showed signals at 2.4 ppm integrated for one proton present in the ring appears as multiplet. –OCH_3 proton of 15th carbon atom comes to resonate at _ 2.904ppm, –OCH3 proton of 16th carbon atom resonate at 3.760 as singlet and -OCH3 of 17th carbon atom resonate at 2.904 as singlet. The aliphatic proton of 7th and 8th carbon atom(C=C) resonates at 6.973 ppm as singlet represent two protons. Finally, the structure assigned as piperlongmine, this conformation was further supported by MASS spectral studies. It gave molecular ion peak at m/z 318 and confirms the structure piperlongumine.

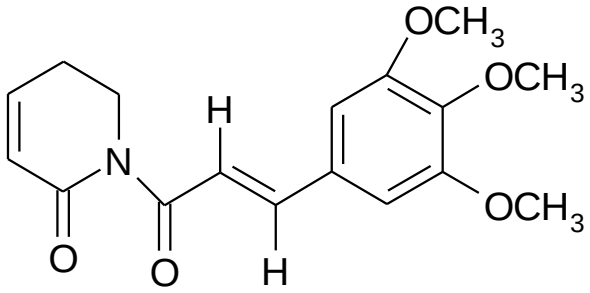


Fig 1: Piperlongumine

All the clinical strains of *Klebsiella pneumoniae* were isolated from the urinary tract infected patients. The constituent piperlongmine was most effective in controlling the growth of these clinical strains and also the MTCC strain (Table 1). The zone of inhibition of the piperlongmine (24.00±0.12 mm) nearer to the value of Ciprofloxacin (24.20±0.12 mm). This showed that piperlongmine is more effective in controlling the growth of *Klebsiella pneumoniae* at 100 µg concentrations when compared to standard antibiotic Ciprofloxacin.

Among different clinical strains of *Pseudomonas aeruginosa* tested for antibiotic activity, the strain isolated from urinary tract infected patients showed maximum zone of inhibition and the antibiotic activity of the constituent piperlongmine is significant (20.00±0.12 mm**)** when the value is compared

with the standard antibiotic Ciprofloxacin. The zone of inhibition of the remaining clinical strains is depicted in Table 1.

The antibacterial effect of the constituent piperlongmine on the colonies of *Staphylococcus aureus* was moderate. Maximum zone of inhibition was observed only in the strains isolated from the pus sample (16.47±0.18 mm) but it is less than the value of the standard antibiotic Ciprofloxacin (21.87±0.47 mm). The zone of inhibition is also moderate in the ATCC strain of *Staphylococcus aureus* (Table 1).

DISCUSSION

Plants are the storehouses for the array of phytochemical constituents for pharmaceutical industry. In most of the populated countries like China and India, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. In Indian system of medicine, the root and fruit of *Piper longum* has been used for curing infectious diseases. The pharmacological property of piperlongumine was screened for Antiplatelet activity[19], and anticancer activity.[20] In the present investigation, piperlongumine was isolated from the methanol extract of the roots and it was characterized by subjecting to ^{1}H NMR and MASS spectral analysis. The constituent piperlongumine were concomitantly tested for antibacterial activity**.** Reports revealed that many plant extracts and the constituents have been subjected to rigorous screening of the biological activity against gram positive and gram-negative bacteria.[21,22]

Klebsiella pneumoniae and *Pseudomonas aeruginosa* were the gram-negative opportunistic pathogens that cause urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a variety of systemic infections. The gram-positive *Staphylococcus aureus* causes a variety of suppurative (pus forming) infections and toxinoses in humans. It also causes superficial skin lesions such as boils and also more serious infections such as pneumonia, mastitis, phlebitis and meningitis. Reports indicated that clinical isolates from different infectious sources from hospitals showed resistance against the drug Methicillin.[23,24] The search for new antimicrobial agents is an important line of research because of the resistance to drugs acquired by the microorganisms.

The results of this investigation revealed that the piperlongmine showed the potent antibacterial activity against the clinical strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from different infectious sources. The antibacterial activity of piperlongmine was most significant against *Klebsiella pneumoniae*, which is a common pathogen in urinary tract infections. The zone of inhibition of the colony was almost equal to that of the standard antibiotic Ciprofloxacin. This supports the traditional use of *Piper longum* in the treatment of microbial infections.[25]

Among all, Gram-negative bacteria, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were more susceptible to piperlongmine than Gram-positive bacterium *Staphylococcus aureus.* This observation contradicts the earlier reports that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria.[26,27] The understanding of the inhibitory mechanism of the isolated active constituent piperlongmine would provide better directions towards the development of efficient production and application of technologies associated with bactericidal plant metabolites.

CONCLUSION

Piperlongmine an isolated constituent have demonstrated antibacterial activity against

clinical strains of selected microorganisms. The isolated constituent piperlongmine shows activity profile. As the crude extract is mixture of several constituents, the purity and the concentration of the isolated constituent exert better activity profile than crude extract. The basis of varying degree of sensitivity of test organism is due to the intrinsic tolerance of microorganism and the chemical nature and structure of the constituent for the mode of action on the control of growth of microorganism is beneficial. Roots and fruits of the plant have been used as a food material in Asian countries; hence the isolated constituent is useful to develop the molecules against infectious diseases. Piperlongmine has shown the better activity profile against gram negative and gram positive bacteria and hence it is a best target for further research for the development of broad spectrum antibacterial agents.

Table 1: Antibacterial activity of Piperlongumine against clinically important bacterial strains.

Bacterial strains tested and source	Piperlongumine			Ciprofloxacin	F-value
	25µg	50 µg	100 µg		
Kp1 Urine	18.00±0.12	20.53±0.29	22.53±0.29	23.40±0.31	83.6
Kp2 Urine	18.40±0.23	21.07±0.18	22.73±0.37	22.87±0.18	68.3
Kp3 Urine	18.27±0.29	20.73±0.29	**24.00±0.12**	23.80±0.12	152.9
Kp4 Urine	18.27±0.24	20.53±0.18	22.93±0.58	23.53±0.18	50.8
Kp5 Urine	18.27±0.18	**21.20±0.12**	23.27±0.37	**24.20±0.12**	141.4
Kp6 MTCC 618	**18.53±0.18**	20.73±0.37	22.60±1.30	23.27±0.18	9.5
Pa1 Urine	14.47±0.24	17.60±0.23	**20.00±0.12**	24.53±0.29	344.9
Pa2 Ear swab	14.73±0.18	17.47±0.24	19.80±0.12	24.27±0.18	489.2
Pa3 Pus	15.07±0.18	**18.07±0.18**	19.87±0.18	23.60±0.31	272.6
Pa4 Stool	15.00±0.12	17.73±0.24	19.20±0.12	**24.73±0.37**	302.4
Pa5 Sputum	14.53±0.18	17.93±0.18	19.60±0.31	23.00±0.12	293.9
Pa6 ATCC 20852	**15.60±0.31**	17.20±0.12	19.33±0.18	22.80±0.12	256.5
Sa1 Wound swab	9.80±0.12	12.20±0.12	15.67±0.18	20.53±0.79	128.6
Sa2 Mucus	**10.00±0.12**	12.80±0.12	16.00±0.12	21.67±0.18	1411.0
Sa3 Hospital effluent	9.53±0.18	12.60±0.12	15.33±0.18	20.33±0.33	449.9
Sa4 Pus	9.60±0.23	12.73±0.18	**16.47±0.18**	**21.87±0.47**	334.0
Sa5 Pimples	9.87±0.24	13.00±0.12	15.87±0.47	21.73±0.64	146.9
Sa6 ATCC 29737	9.27±0.18	**13.00±0.31**	15.33±0.24	19.67±0.33	258.6

The values are the mean of three experiments ± S.E.
The F-value is significantly different at the 0.05% probability level
Abbreviations: Pa, *Pseudomonas aeruginosa*; Kp, *Klebsiella pneumoniae*; Sa, *Staphylococcus aureus*

REFERENCES

1. Roja, G and Rao, PS (2000), "Anticancer compounds from tissue cultures of medicinal plant", ***Journal of Herbs, Spices & Medicinal Plants***, Vol. 7, 71-102.
2. Awadh Ali, NA; Juelich, WD; Kusnick, C and Lindequist U (2001), "Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities", ***Journal of Ethnopharmacology,*** Vol. 74, 173-179.

3. Nitta, T; Arai, T; Takamatsu, H; Inatomi, Y *et al.* (2002),"Antibacterial activity of extracts preparated from tropical and subtropical plants on methicillin- resistant *Staphylococcus aureus*", ***Journal of Health Science***, Vol. 48, 273-276.
4. Ahmad, I; Mehmood, Z and Mohammad, F (1998), "Screening of some Indian medicinal plants for their antimicrobial properties", ***Journal of Ethnopharmacology,*** Vol. 62, 183-193.
5. Kameshwara Rao, C (2000), "***Databases of Medicinal Plants****"*, Karnataka State Council for Science and Technology Publisher, Bangalore, India, 1-23.
6. Subramani, SP and Goraya, GS (2003), "Some Folklore medicinal plants of Kolli hills: Record of a Watti Vaidyas sammelan", ***Journal of Economic and Taxonomic Botany***, Vol. 27, 665-678.
7. Shahidi, GH and Karimi Nik, A (2004), "Antibacterial activity of some medicinal plants of Iran against *Pseudomonas aeruginosa* and *P. fluorescens*", ***Asian Journal of Plant Sciences***, Vol. 3, 61-64.
8. Basile, A; Giordano, S; Lopez-Saez, JA and Cobianchi, RC (1999), "Antibacterial activity of pure flavonoids isolated from mosses", ***Phytochemistry***, Vol. 52, 1479-1482.
9. Cragg, GM and Newman, DJ (2001), "Natural product drug discovery in the next millennium", ***Pharmaceutical Biology***, Vol. 39 (1), 8-17.
10. Wink, M (1999), "Biochemistry of Secondary Product Metabolism", ***Introduction Biochemistry, role and biotechnology of secondary products***, CRC Press, Boca Raton, Florida, 1-16.
11. Yoganarasimhan, SN (1996), "***Medicinal plants of India***", Interline publishers, Bangalore, India, 366.
12. Kirtikar, KR and Basu, BD (1984), "***Indian Medicinal Plants***", Periodical Expert Book Agency, New Delhi, India.
13. Warrier, PK; Nambiar, VPK and Raman, KC (1995), "***Indian Medicinal Plants***", Vol. 4, Orient Longman Ltd, Madras, India.
14. Cowan, ST and Steel, S (1993), "***Manual for the Identification of Medica Bacteria***", Cambridge University Press, England, 32.
15. Aneja, KR (2003), "***Experiments in Microbiology, Plant Pathology and Biotechnology***", New Age International Ltd., New Delhi, India, 196-197.
16. Nair, R; Kalariya, T and Chanda, S (2005), "Antibacterial activity of some selected Indian medicinal flora", ***Turkish Journal of Biology***, Vol. 29, 41-47.
17. Collins, CH; Lyne, PM and Grange, JM (1989), "***Microbiological Methods***", 6th Edition, Butterworhs Co. Ltd., London, 410.
18. Ali-Shtayeh, MS; Yaghmour, RM; Faidi, YR; Salem, K *et al.* (1998), "Antimicrobial activity of 20 plants used in folkloric medicine in the Plastenian area", ***Journal of Ethnopharmacology***, Vol. 60, 265-271.
19. Masaya, I; Nobuaki, O; Satoko, O; Masaki, S *et al*. (2007), "Piperlongumine, a constituent of *Piper longum* L., inhibits rabbit platelet aggregation as a thromboxane A_2 receptor antagonist", ***European Journal of Pharmacology***, Vol. 570, 38-42.
20. Sam, WL and Anna, M (2008), "***Methods for the treatment of cancer using piperlongumine and piperlongumine analogs***", U.S. patent 20090312373.
21. Irvine, FR (1961), "***Woody Plants of Ghana***", Oxford University press, London, 878.
22. Mukhlesur, R and Alexander, IG (2002), "Antimicrobial constituents from the stem bark of *Feronia limonia*", ***Phytochemistry,*** Vol. 59, 73.
23. Waldyogel, FA (1995), "*Staphylococcus*

aureus (including Toxic Shock Syndrome)", ***Principles and Practice of Infectious Disease***, New York. 1754-1777.

24. Chambers, HF (1997), "Methicillin Resistance in *Staphylococci:* Molecular and Biochemical basis and Clinical implications", ***Clinical Microbiology Reviews,*** Vol. 10(4), 781-791.
25. Srinivasa, RP; Kaiser, J; Madhusudhan, P; Anjani, G *et al.* (2001), "Antibacterial activity of isolated from *Piper longum* and *Taxus baccata*", ***Pharmaceutical Biology***, Vol. 39, 236- 238.
26. Vlietinck, AJ; Van Hoof, L; Totte, J; Lasure, A *et al.* (1995), "Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties", ***Journal of Ethnopharmacology***, Vol. 46, 31-47.
27. Rabe, T and Van Staden J (1997), "Antibacterial activity of South African plants used for medicinal purposes", ***Journal of Ethnopharmacology***, Vol. 56, 81-87.