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IN VITRO EVALUATION OF CYTOTOXIC ACTIVITY OF *PIPER LONGUM* L. ROOT (LONG PEPPER) ON SOME IMPORTANT SPECIES OF BACTERIA

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ABSTRACT
In vitro evaluation of antibacterial activity of aqueous and solvent extract of *Piper longum* root was evaluated against five bacterial species viz., *Staphylococcus aureus Escherichia coli, Bacillus subtilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. In aqueous extract tested at 10 to 100% concentration, maximum inhibition was observed in *S. aureus* (31.0mm) and *B. subtilis* (31.0mm) followed by *E. coli* (30.0mm) and *P. aeruginosa* (23.0mm). In 10 to 90% concentration, the inhibition percentage was in the range of 5.0 to 29.0 mm respectively. In solvent extract, high percentage of inhibition was observed in petroleum ether extract and recorded a maximum inhibition of 32.0mm and minimum of 27.0mm in all test bacterial species. Petroleum ether extract was followed by methanol, chloroform and ethanol extract. Compared to synthetic antibiotic xanthomycein, chloramphenicol and streptomycein at 25mg concentration, maximum inhibition of 35.0 mm and minimum of 30.0mm was observed in all the test bacteria. The Minimum Inhibitory Concentration (MIC) was identified for all the test bacterial species.

KEYWORDS: Bacteria, Aqueous extract, Solvent extract, MIC, Synthetic antibiotic.

INTRODUCTION
In recent years, multiple drug resistance in both human and plant pathogens has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases.[1] The multidrug resistant strain of many microorganisms has revealed exploration of alternative antimicrobial agent. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects.[2] Recently, there has been a great deal of attention paid in medical treatments to plant extracts and compounds with biological features, because of the resistance and side effects that the micro-organisms of pathogens have shown in the face of antibiotics.[3] To over, the ill effects of these synthetic drugs, there is an urgent need to search an alternative source**.** The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country.[4] Plant-based antimicrobials represent a vast untapped source of medicines and are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.[5]

Long pepper is grown in South India. It is commonly used to support digestive and respiratory system. *Piper longum* is a close relative of *Piper nigrum* (Black Green and white peppercorns) with a similar but more pungent taste. The root of the plant is called Modi or Pippali-moolam. The root is more suitable for respiratory system. In the present study, *P. longum* (root) belongs to family piperaceae was evaluated for antibacterial activity against five bacterial species.

MATERIALS AND METHODS
Plant Material: Fresh and healthy roots of *P. longum* collected from Mysore. The roots were thoroughly washed two to three times with running tap water and one to two times with sterile distilled water. The washed roots were air dried at room temperature on a sterile

blotter, and used for the preparation of plant and solvent extracts.

Preparation of Aqueous extract

One hundred grams of the thoroughly washed and air dried roots of *P. longum* were macerated with 100 ml of sterile distilled water in a waring blender (Waring International, New Hartford, CT, USA) for five minutes. The macerate was filtered through double-layered muslin cloth, and then centrifuged at 4000g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120^0 C for 10 minutes, which served as 100% aqueous mother extract. The extract was preserved aseptically in a sterile brown bottle at 5^0 C until further use. [6-7]

Preparation of Solvent extraction

Thoroughly washed roots of *P. longum* were dried in shade for five days and then powdered with the help of Waring blender. 25 grams of shade dried powder was filled in the thimble and extracted successively with petroleum ether, chloroform, methanol and ethanol in a Soxhlet extractor for 48 hours. Solvent extracts were concentrated under reduced pressure. After complete evaporation, 1 gram of each concentrated solvent extracts were dissolved in 9 ml of methanol and used for antibacterial assay.[8]

Test organisms

Five species of bacteria viz., *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram Negative), *Bacillus subtilis* (Gram positive), *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Gram Negative) were collected from CMR research center, CMR Institute of Management Studies (Autonomous), Bangalore. The obtained cultures were sub cultured on nutrient agar medium. After 24 hours of incubation at 37°C, the cultures were preserved aseptically in refrigerator until further use.

ANTIBACTERIAL ACTIVITY

Preparation of standard culture inoculums of test organism: Fresh culture of all the test bacterial species were inoculated into 2 ml of Nutrient broth and incubated at 37^0 C for 24 hours till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.

Aqueous extract

Agar cup diffusion method

Agar cup diffusion method described by[9] was employed. An overnight culture of S. aureus, E. coli, B. subtilis, K. pneumonia and P. aeruginosa was standardized to contain approx.107cfu/ml and inoculated into 20 ml of nutrient broth. And allowed to set for 30 minutes and all the inoculum was swabbed over the surface of nutrient agar medium plate using sterilized spreader. Sterilized cork borer of 5 mm diameter was taken and, five wells were made in solidified sterile nutrient agar medium plate (one in the centre and four wells at the corner). The agar plugs were removed with a flamed and cooled wire loop and 10,20,30,40, 50, 60, 70, 80, 90 and 100µl of aqueous extract of *P. longum* roots was added to the wells made in inoculated plates. The treatment also includes 50 µl of absolute alcohol served as control. All the plates were incubated for 24 hours at 37^oC and zone of inhibition if any around the well were measured in millimeter (mm). For each treatment ten replicates were maintained. The same procedure were followed for standard antibiotics xanthomycin (25mg), streptomycin (25mg) and chloramphenicol (25mg) to compare the efficacy of plant extract against test organisms.

Solvent Extract

One gram of different solvent extract of *P. longum* roots were dissolved in 9 ml of methanol. The sterile nutrient agar medium in petridishes was uniformly smeared with test culture. 5 mm wells were made in each petridish to which 50µl of 500, 1000, 1500 and 2000ppm of different solvent extracts dissolved in methanol were added. For each treatment ten replicates were maintained. Respective solvents served as control. Standard antibiotics viz., xanthomycin (25mg), streptomycin (25mg) and chloramphenicol (25mg) was used to compare the efficacy of solvent extract against test organisms.[8]

STATISTICAL ANALYSIS

The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05).

RESULT AND DISCUSSION

Antibacterial activity of aqueous extract

Among the five bacterial species tested, *S. aureus* recorded a maximum inhibition of 31.0mm at 80% concentration and recorded MIC at 80%. *B. subtilis* showed a maximum inhibition at 90 and 100% concentration and recorded 30.0 mm and 31.0mm respectively. *E. coli* recorded 30.0mm inhibition at 100% concentration and *P. aeruginosa* recorded 30.0mm inhibition at and MIC at 90% concentration. In *K. pneumonia*, significant activity was observed at 80% concentration and recorded 23.0mm inhibition. From 10 to 70% concentration, in all the test bacterial species, the inhibition percentage was ranged from 5.0mm to 29.0mm in *S. aureus*, 4.0mm to 26.0mm in *B. subtilis* , 6.0mm to 25.0mm in *E. coli* , 5.0mm to 27.0mm in *P. aeruginosa* and 2.0mm to 21.0mm in *K. pneumonia* (Table 1). Compared to synthetic antibiotic xanthomycin, chloramphenicol and streptomycein at 25mg concentration, all the test bacterial species recorded the inhibition percentage form 30.0mm to 33.0mm respectively.

Antibacterial activity of solvent extract

Four solvent extract viz., petroleum ether extract, chloroform, methanol and ethanol were treated at 500, 1000, 1500 and 2000ppm concentration. Among the four solvent extract tested, petroleum ether extract recorded a maximum inhibition against all the test bacteria at 2000ppm, *S. aureus* recorded 31.0mm inhibition and at 500ppm, maximum inhibition of 14.0mm. *B. subtilis* recorded 32.0mm and 11.0mm inhibition at 2000ppm and 500ppm respectively. *E. coli* recorded 31.0 and 10.0mm, *K. pneumonia* recorded 27.0mm and 6.0mm and *P. aeruginosa* recorded 29.0mm and 7.0mm inhibition respectively.

Petroleum ether was followed by methanol extract and recorded maximum inhibition in *S. aureus* with 28.0mm at200ppm and 12.0mm at 500ppm concentration. *E. coli* showed 27.0mm and 10.0mm inhibition at 2000ppm and 500ppm respectively. *B. subtilis* recorded 25.0mm and 10.0mm inhibition at 2000ppm and 500ppm concentration. *K. pneumonia* and *P. aeruginosa* showed 23.0mm and 20.0mm inhibition at 2000ppm and 500ppm concentration.

In chloroform extract, maximum inhibition was observed in *E. coli* and *P. aeruginosa* and recorded 22.0mm inhibition at 2000ppm concentration. *S. aureus* and *B. subtilis* recorded 20.0mm at 2000ppm concentration. Significant activity was observed in *K. pneumonia* and recorded 17.0mm inhibition. Maximum activity was observed at 500, 1000 and 1500ppm concentration in the range of 5.0mm to 23.0mm inhibition respectively.

Least inhibition was observed in ethanol extract and recorded 14.0, 14.0, 13.0, 12.0 and 9.0mm inhibition at 2000ppm concentration and 500, 1000 and 1500 ppm concentration. At 500, 1000 and 1500ppm concentration, the inhibition percentage is in the range of 1.0 to 12.0 mm.

Compared to synthetic fungicides, xanthomycin, chloramphenicol and streptomycin at 25mg concentration, maximum inhibition of 33.0mm and minimum inhibition of 27.0 mm was observed in all the test bacterial species.

Medicinal plants have been used to treat common infectious diseases for centuries and some of them are the source of traditional medicines.[10] The use of plant extracts and photochemical both with known antimicrobial properties are of great significance. The plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. Which have been found to have vast antimicrobial properties in vitro.[11-12] Medicinal plant parts have been extensively used to extract raw drugs owing to possession of various medicinal properties. They constitute credible sources for a huge number of modern drugs, several of which are usually based on their traditional folk medicine.[13] The World Health Organization (WHO) has stated that medicinal plants are the best source for obtaining a variety of therapeutic agents, and several medicinal plants have been employed as a source of medicine in daily life for treatment of various types of ailments globally.[14] From the above result, in aqueous extract, *S. aureus* and *B. subtilis* recorded a maximum activity in less concentration. *E.coli* also recorded a significant activity in low concentration of the aqueous extract. Among the different solvent extracts, petroleum ether and methanol recorded an maximum inhibition and the results were equal to synthetic antibiotics tested. In chloroform and methanol extract, moderate activity was observed in different concentration of solvent tested.

Table 1: Antibacterial activity of aqueous extract of *P. longum* roots (Long pepper)

Bacteria	Zone of inhibition(mm)													
	Concentration													
	Plant extract											Synthetic antibiotics		
	10 %	20 %	30 %	40%	50%	60%	70%	80%	90%	100%	MIC	Xanthomycin (25mg)	Chloramphenicol (25mg)	Streptomycin (25mg)
S. aureus	5.0^{a} ±0.1	10.0^{b} ±0.1	15.0^{c} ±0.0	21.0^{d} ±0.1	23.0^{e} ±0.0	26.0^{f} ±0.0	29.0^{g} ±0.0	31.0^{h} ±0.0	31.0^{h} ±0.0	31.0^{i} ±0.0	80%	32.0^{c} ±0.1	31.0^{b} ±0.1	30.0^{a} ±0.1
E. coli	6.0^{a} ±0.1	9.0^{b} ±0.2	13.0^{c} ±0.0	17.0^{d} ±0.0	20.0^{e} ±0.1	23.0^{f} ±0.1	25.0^{g} ±0.1	27.0^{h} ±0.0	29.0^{i} ±0.1	30.0^{j} ±0.1	100%	31.0^{b} ±0.2	33.0^{c} ±0.2	30.0^{a} ±0.2
B. subtilis	4.0^{a} ±0.2	8.0^{b} ±0.0	12.0^{c} ±0.0	17.0^{d} ±0.0	19.0^{e} ±0.0	22.0^{f} ±0.0	26.0^{g} ±0.1	29.0^{h} ±0.1	30.0^{i} ±0.0	31.0^{i} ±0.0	100%	33.0^{c} ±0.1	31.0^{b} ±0.1	27.0^{a} ±0.1
K. pneumonia	2.0^{a} ±0.1	4.0^{b} ±0.0	9.0^{c} ±0.0	13.0^{d} ±0.1	17.0^{e} ±0.0	20.0^{f} ±0.1	21.0^{g} ±0.0	23.0^{h} ±0.0	23.0^{h} ±0.1	23.0^{h} ±0.0	80%	30.0^{a} ±0.3	32.0^{c} ±0.2	31.0^{b} ±0.1
P. aeruginosa	5.0^{a} ±0.1	9.0^{b} ±0.0	14.0^{c} ±0.0	18.0^{d} ±0.0	21.0^{e} ±0.0	24.0^{f} ±0.0	27.0^{g} ±0.1	29.0^{h} ±0.0	30.0^{i} ±0.0	30.0^{i} ±0.1	90%	30.0^{a} ±0.2	31.0^{b} ±0.1	33.0^{c} ±0.0

- Values are the mean of ten replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

Table 2: Antibacterial activity of solvent extracts of *P. longum* roots (Long pepper)

Bacteria	Zone of inhibition(mm)										
	Concentration										
	Ethanol extract				Methanol extract				Synthetic antibiotics		
	500 ppm	1000ppm	1500ppm	2000 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm	Xanthomycin (25mg)	Chloramphenicol (25mg)	Streptomycin (25mg)
S. aureus	2.0^{a} ±0.0	6.0^{b} ±0.0	9.0^{c} ±0.0	14.0^{d} ±0.1	12.0^{a} ±0.0	16.0^{b} ±0.1	21.0^{c} ±0.0	28.0^{d} ±0.1	32.0^{c} ±0.1	31.0^{b} ±0.1	30.0^{a} ±0.1
E. coli	3.0^{a} ±0.0	7.0^{b} ±0.0	10.0^{c} ±0.1	13.0^{d} ±0.0	10.0^{a} ±0.0	15.0^{b} ±0.0	20.0^{c} ±0.1	27.0^{d} ±0.0	31.0^{b} ±0.2	33.0^{c} ±0.2	30.0^{a} ±0.2
B. subtilis	2.0^{a} ±0.1	5.0^{b} ±0.1	12.0^{c} ±0.0	14.0^{d} ±0.1	10.0^{a} ±0.1	18.0^{b} ±0.0	23.0^{c} ±0.0	25.0^{d} ±0.0	33.0^{c} ±0.1	31.0^{b} ±0.1	27.0^{a} ±0.1
K. pneumonia	1.0^{a} ±0.0	4.0^{b} ±0.0	7.0^{c} ±0.1	12.0^{d} ±0.1	5.0^{a} ±0.0	12.0^{b} ±0.1	18.0^{c} ±0.0	23.0^{d} ±0.1	30.0^{a} ±0.3	32.0^{c} ±0.2	31.0^{b} ±0.1
P. aeruginosa	1.0^{a} ±0.0	3.0^{b} ±0.1	5.0^{c} ±0.0	9.0^{d} ±0.0	8.0^{a} ±0.1	12.0^{b} ±0.0	16.0^{c} ±0.1	20.0^{d} ±0.1	30.0^{a} ±0.2	31.0^{b} ±0.1	33.0^{c} ±0.0

- Values are the mean of ten replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.

- Pattern of percentage inhibition increase is not uniform for all the microorganisms

Table 3: Antibacterial activity of solvent extracts of *P. longum* roots (Long pepper)

Bacteria	Zone of inhibition(mm)										
	Concentration										
	Petroleum ether extract				Chloroform extract				Synthetic antibiotics		
	500 ppm	1000ppm	1500ppm	2000ppm	500 ppm	1000ppm	1500 ppm	2000 ppm	Xanthomycin (25mg)	Chloramphenicol (25mg)	Streptomycin (25mg)
S. aureus	14.0^{a} ±0.1	21.0^{b} ±0.0	27.0^{c} ±0.1	31.0^{d} ±0.0	8.0^{a} ±0.0	13.0^{b} ±0.1	15.0^{c} ±0.0	20.0^{d} ±0.0	31.0^{c} ±0.1	30.0^{b} ±0.1	30.0^{a} ±0.1
E. coli	10.0^{a} ±0.1	16.0^{b} ±0.1	23.0^{c} ±0.0	31.0^{d} ±0.1	9.0^{a} ±0.1	14.0^{b} ±0.0	19.0^{c} ±0.1	22.0^{d} ±0.1	35.0^{c} ±0.2	32.0^{b} ±0.2	30.0^{a} ±0.2
B. subtilis	11.0^{a} ±0.0	19.0^{b} ±0.1	28.0^{c} ±0.1	32.0^{d} ±0.0	4.0^{a} ±0.0	11.0^{b} ±0.1	16.0^{c} ±0.0	20.0^{d} ±0.0	32.0^{b} ±0.1	32.0^{c} ±0.1	27.0^{a} ±0.1
K. pneumonia	6.0^{a} ±0.0	13.0^{b} ±0.1	19.0^{c} ±0.0	27.0^{d} ±0.1	3.0^{a} ±0.0	8.0^{b} ±0.0	13.0^{c} ±0.1	17.0^{d} ±0.1	30.0^{a} ±0.3	33.0^{c} ±0.2	31.0^{b} ±0.1
P. aeruginosa	7.0^{a} ±0.1	15.0^{b} ±0.0	22.0^{c} ±0.1	29.0^{d} ±0.0	5.0^{a} ±0.1	11.0^{b} ±0.0	16.0^{c} ±0.0	22.0^{d} ±0.0	32.0^{c} ±0.2	30.0^{a} ±0.1	33.0^{b} ±0.0

- Values are the mean of ten replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

CONCLUSION

From the above observation and result, it can be concluded that, *P. longum* roots showed a promising result in aqueous and solvent extract against all the test bacteria . compared to synthetic antibiotics, *P. longum* roots extract also recorded a maximum inhibition. Hence a further investigation is necessary to isolate the active principles responsible for antibacterial activity and its characterization which is ecofriendly and easily biodegradable.

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REFERENCE

1. BA Jamuna; RV Ravishankar; VS Pradeepa, Malaysian J Microbiol., 2011; 7(1): 14-18.
2. Bhushan Bhaskarwar; Prakash Itankar; Abhay Fulke**,** Roumanian Biotechnological Letters., 2008; 13(5): 3873-3877.
3. Somayeh Jahani; Saeide Saeidi; Fereshteh Javadian ; Zahra Akbarizadeh; Ali Sobhanizade, Int J Infect., 2016; 3(2): 34081.
4. Bishnu joshi; Sunil Lekhak; Anuja Sharma, Kathmandu University Journal of Science, Engineering And Technology., 2009; 5(1): 143-150.
5. J Parekh; D Jadeja; S Chanda, Turk. J. Biol., 2005; 29: 203-210.
6. GGF Nascimento; J Locatelli; PC Fereitas; Silva GL. Braz. Journal of Microbiology., 2000; 31: 247-256.
7. B Kiran; V Lalitha; KA Raveesha, World Journal of Pharmaceutical research, 2015; 1(4): 1077-1083.
8. V Lalitha; B Kiran; KA Raveesha, Pharmacophore, 2011; 2(1): 78-85.
9. SB Bole; R Manju; M Nagaraj; V Sandhya ; G Supriya; Pranitha Kumari ;B Kiran; V Lalitha, Pharmacophore, 2010; 1(3): 178-183.
10. DK Raja; NS Jeganathan; R Manavalan, International Current Pharmaceutical Journal, 2013; 2(6): 105-108.
11. MM Cowan, Clinical microbiology reviews, 1999; 12: 564-82.
12. RD Surekha; B Shankar Naik, Journal of Applied Pharmaceutical Science, 2016; 6(01); 83-85.
13. Ayad Ismael Khaleel; Kamaruzaman Sijam; Tavga Sulaiman Rashid; Khairulmazmi Bin Ahmad, American Journal of Plant Sciences., 2016; 7: 159-166.
14. MN Alo; C Anyim; J C Igwe; M Elom; DS Uchenna, Adv. Appl. Sci. Res., 2012; 3: 844-848.