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In-vitro studies on bacteriostatic effects of solvent extracts of Kodithotakalli leaves of *Piper betel* L. Cv. Kapoori -A local cultivar

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ABSTRACT

India, being one amongst the 12 Mega- Bio-diverse countries of the world, is abode of a wide variety of medicinal plants. It is evident that the Indian people have colossal passion for medicinal plants and they use them for wide range of health related applications. 1500 medicinal plants have been tagged out by Indian systems, of which 500 species are commonly used in the preparation of herbal drugs. Of all plant drugs, Indian drugs contribute as much as 80%. Drugs of herbal origin have been used in customary medicines such as *Unani* and *Ayurveda*, found to be widely exercised on many accounts. Most of the drugs given even today are directly or indirectly from natural sources .Herbal medicines are safe, free from side effects and eco-friendly. As a result, herbal medicine has gained momentum and it is evident from the fact that certain herbal remedies are more effective as compare to synthetic drugs. In consonance with this information, *Piper betel* L. (Green gold of India), which is commonly considered as a traditional medicinal plant, was choosen for the study. Kodithotakalli leaves of *Piper betel* L. Cv. Kapoori were solvent extracted in ether, chloroform, ethanol and methanol, tested for antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Corynebacterium diphtheria*, *Xanthomonas citrovorum*, *Proteus vulgaris* and *Staphylococcus aureus*. The ether extracts displayed high activity against tested bacteria even at lowest concentrations in comparison with other solvent extracts.
Keywords: *Piper betel* L. Cv. Kapoori, Kodithotakalli leaves, solvent extracts and antibacterial activity.

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INTRODUCTION

The mileage of plants as medicines is as ancient as human civilization itself. A myriad of plants have been in use by the active systems of medicine such as Ayurveda, Unani, Homeopathy, Naturopathy, Siddha and other alternative medicinal systems to cure many diseases. India being the largest producer of medicinal herbs is appropriately called the Botanical Garden of the World (Ahmedulla and Nayer, 1999). Piper betel L. (Green gold of India) belongs to the family Piperaceae. Piper betel L. (Green gold of India) is a climber with broad, heart-shaped, dark green and shiny, juicy leaves. It is used as an ornamental plant and is also known for its medicinal properties like antimicrobial, antioxidant, stimulant and for arthritis (Kirtikar and Basu, 1998). The betel leaf plant is branching slender stemmed vine, climbing as high as 10-15 feet (Prajapathi, 2003). The principal aim of the work was to study the antibacterial activity of Kodithotakalli leaf extracts of Piper betel L. Cv. Kapoori in different solvents such as ethanol, chloroform, methanol and petroleum ether against seven species of bacteria. In the experimental study, different fractions of solvent extracts of Kodithotakalli leaves of the Piper betel L. Cv. Kapoori, a local cultivar have been investigated.

PLANT MATERIAL

Plant collection, identification and authentication

Kodithotakalli leaves of Piper betel L. Cv. Kapoori (a local cultivar) were collected from Chintalapudi, Ponnur, Guntur district of Andhra Pradesh, India, in January 2011. The plant was identified and authenticated by the taxonomist of Faculty of Botany, Hindu college, Guntur and a voucher specimen (ANU/PB/2011-003) was deposited at Department of Botany, Acharya Nagarjuna University, Nagarjuna nagar, Guntur, Andhra Pradesh, India for future reference.

Extract Preparation

Fresh plant material was washed thoroughly under tap water, shade dried and used for extraction. The dried leaves were homogenized to a fine powder and stored in airtight bottles. 25g of leaf powder was extracted with 150 ml of solvent (chloroform, ethanol, ether and methanol) for 24h using Soxhlet apparatus. The extract was dried in a flash evaporator for 30min and the left over powder was considered 100%. Different concentrations such as 250µg/ml, 500µg/ml, 750µg/ml and 1000µg/ml were prepared by redisolving the extract powder in the same solvent that was used for extraction (S. H. K. R. Prasad et al. 2011).

Test Organisms

Escherichia coli (ATCC 25922), Bacillus subtilis (ATCC 6633), Pseudomonas aeruginosa (ATCC 27853), Corynebacterium diphtheria (ATCC 75415), Xanthomonas citrovorum (ATCC 8082), Proteus vulgaris (ATCC 638) and Staphylococcus aureus (ATCC 25923) obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Hindu College of Pharmacy,

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Guntur, Andhra Pradesh (Characteristics are listed in Table No. 1) were used in the current study. All the above test bacterial species were maintained on Nutrient Agar medium.. 36hr-old bacterial culture was inoculated into Nutrient broth and incubated on a rotary shaker at 35 ± 2°C at 100 rpm. After 36 hours of incubation, the bacterial suspension was centrifuged at 10000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1×10^8 cfu/ml using UV Visible Spectrophotometer. By reading the OD of the solution to 0.45Å (610nm) it was used for further studies (K. Anthonamma et al. 2010).

Table No 1: List of the selected test organisms (Bacteria)

S.NO	NAME OF ORGANISM	CHARACTERISTICS FEATURES	DISEASE CAUSED BY ORGANISM
1.	*Escherichia Coli* (ATCC 25922)	Gram -ve rod shaped organism	Gastroenteritis
2.	*Bacillus subtilis* (ATCC 6633)	Gram +ve rod shaped organism	Food poisoning
3.	*Pseudomonas aeruginosa* (ATCC 27853)	Gram –ve rod shaped organism	Wounds and urinary tract infections
4.	*Corynebacterium diphtheria*(ATCC 75415)	Gram +ve rod shaped organism	Diphtheria
5.	*Xanthomonas citrovorum* (ATCC 8082)	Gram –ve rod shaped organism	Urinary tract infections
6.	*Proteus vulgaris* (ATCC 638)	Gram negative rod-shaped bacterium	Urinary tract infections and wound infections
7.	*Staphylococcus aureus* (ATCC 25923)	Facultative anaerobic, gram-positive coccus	Minor skin infections, pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS)

Antibacterial Assay

Different concentrations of solvent extracts of the leaves were tested for antimicrobial activity by using Antibiotic Sensitivity test (Barry, A.C. 1976, Benson, H.J. 1990). Bacterial suspension was evenly mixed with sterile Agar medium and poured into sterile Petri plates. After allowing the media to solidify at room temperature, wells of 6mm diameter were bored in the agar with sterile cork borer. Each concentration was checked for antibacterial activity by introducing equal amounts of the sample (40µl) into wells. The method was repeated in five plates. Plates were allowed to stand at room temperature for 1 hour, for extract to diffuse into agar media and then incubated at 37 °C for 24 to 48 hours. The zone of growth inhibition around the wells was measured and diameter of inhibition zone was calculated. Simultaneously, the activity of standard antibiotic Streptomycin (10µg/ml) was studied under similar conditions, so as to compare the degree of inhibition by the solvent extracts. Agar wells fed with corresponding solvents served as control. Minimum Inhibitory Concentration which was determined as the lowest concentration of solvent extracts inhibiting the growth of organisms, was determined based on the readings.

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RESULTS

The results of the study are given in Table 2 and graphical representations of the each solvent given separately as Graph 1, 2, 3 and 4. The results indicate that the ether extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori were inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 250µg/ml for all tested bacteria such as Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Corynebacterium diphtheria, Xanthomonas citrovorum, Proteus vulgaris and Staphylococcus aureus. Among the bacterial species tested Corynebacterium diphtheria was found to be highly sensitive to the ether extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori, a local cultivar (Graph 1). The chloroform extracts of the Kodithotakalli leaves of Piper betel L. Cv. Kapoori were also inhibitory to all the test organisms (Graph 2). The minimum inhibitory concentration of chloroform extracts was found to be 500µg/ml for Corynebacterium diphtheria, while it was 750µg/ml for Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Xanthomonas citrovorum, Proteus vulgaris and Staphylococcus aureus. Proteus vulgaris was found to be highly sensitive to the chloroform extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori, of all the bacterial species tested. All the test organisms were found to be susceptible to the ethanol extracts of the Kodithotakalli leaves of Piper betel L. Cv. Kapoori. The minimum inhibitory concentration was found to be 500µg/ml for Escherichia coli, Bacillus subtilis, Corynebacterium diphtheria, Proteus vulgaris and Staphylococcus aureus, while it was 750µ/ml for Pseudomonas aeruginosa and Xanthomonas citrovorum. Among the bacterial species tested, Corynebacterium diphtheria was found to be highly susceptible to the ethanol extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori (Graph 3). The methanol extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori also inhibited all the test organisms (Graph 4). The minimum inhibitory concentration was found to be 500µg/ml for Escherichia coli, Bacillus subtilis, Corynebacterium diphtheria and Staphylococcus aureus. The remaining three bacteria exhibited zones of growth inhibition at 750µg/ml. Of all the test organisms, Staphylococcus aureus was found to be highly sensitive to the methanol extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori, a local cultivar.

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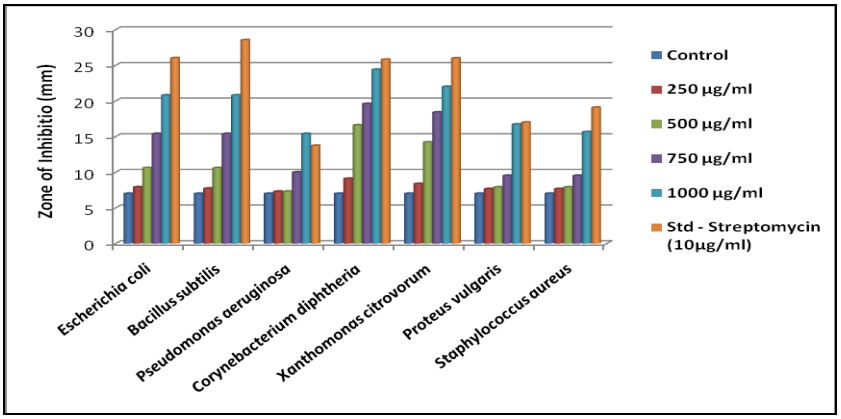
Table No 2: Inhibitory activity of solvent extracts of Kodithotakalli leaves of *Piper betel* L. Cv. Kapoori (a local cultivar).

Solvent extract	Product (µg)	Zone of Inhibition (mm)						
		A	B	C	D	E	F	G
Ether	Control	7.00	7.00	7.00	7.00	7.00	7.00	7.00
	250	7.90	7.72	7.30	9.10	8.38	7.66	7.66
	500	10.6	10.6	7.30	16.6	14.2	7.90	7.90
	750	15.4	15.4	10.00	19.6	18.4	9.52	9.52
	1000	20.8	20.8	15.4	24.4	22.00	16.72	15.65
Chloroform	Control	9.00	9.00	9.00	9.00	9.00	9.00	9.00
	250	9.00	9.00	9.00	9.00	9.00	9.00	9.00
	500	9.00	9.00	9.00	10.00	9.00	9.00	9.00
	750	11.4	11.4	14.4	13.2	13.2	12.6	12.6
	1000	14.4	14.4	17.4	17.4	16.8	17.14	16.45
Ethanol	Control	9.00	9.00	9.00	9.00	9.00	9.00	9.00
	250	9.00	9.00	9.00	9.00	9.00	9.00	9.00
	500	10.2	10.2	9.00	10.2	9.00	9.60	9.72
	750	13.8	13.8	11.4	14.4	11.4	10.8	16.45
	1000	17.4	17.4	13.80	19.20	13.80	12.96	17.65
Methanol	Control	8.60	8.60	8.60	8.60	8.60	8.60	8.60
	250	8.60	8.60	8.60	8.60	8.60	8.60	8.60
	500	8.84	8.84	8.60	11.24	8.60	8.60	10.76
	750	10.64	10.64	8.84	13.64	8.84	11.00	16.34
	1000	13.04	13.04	11.24	17.84	11.84	11.00	26.24
Std*		26.03	28.55	13.73	25.81	26.00	17.00	19.08

*Standard: Streptomycin (10µg/ml) for Bacteria

A) *Escherichia coli* B) *Bacillus subtilis* C) *Pseudomonas aeruginosa* D) *Corynebacterium diphtheria* E) *Xanthomonas citrovorum* F) *Proteus vulgaris* G) *Staphylococcus aureus*



Graph 1: Inhibitory activity of ether extracts of Kodithotakalli leaves of *Piper betel* L. Cv. Kapoori, a local cultivar.

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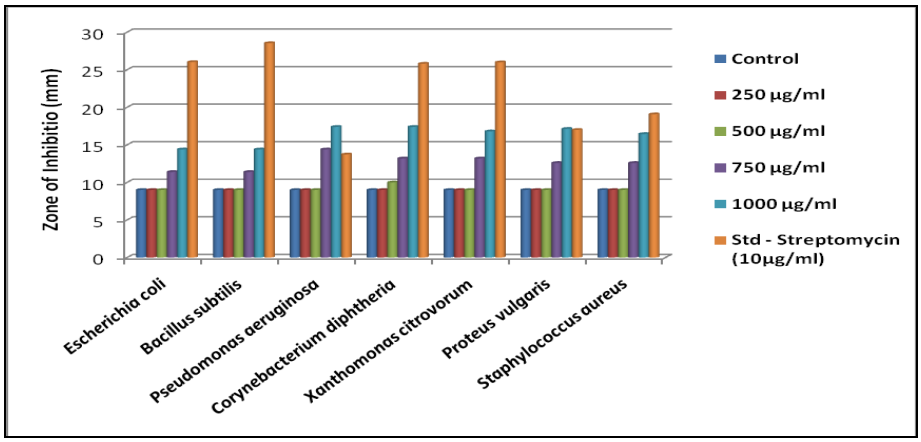
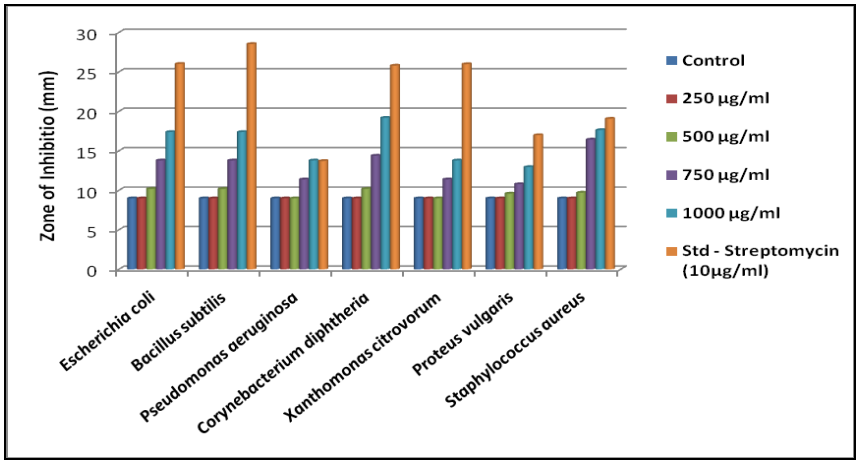
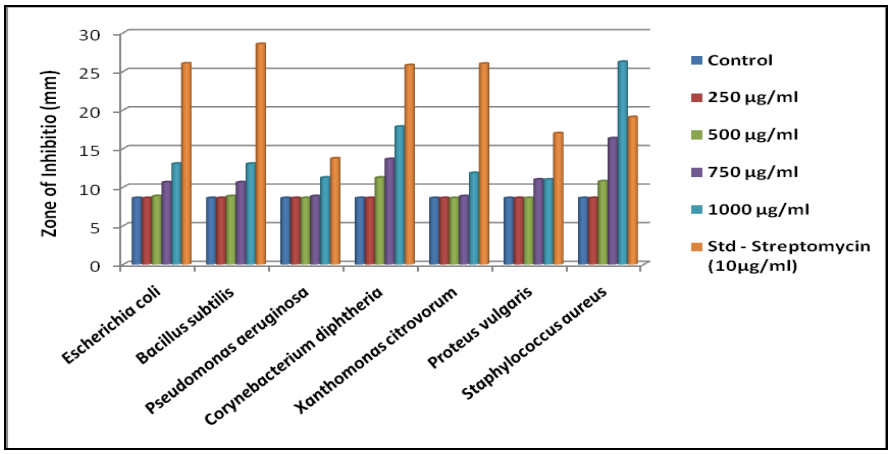


Graph 2 Inhibitory activity of chloroform extracts of Kodithotakalli leaves of *Piper betel* L. Cv. Kapoori, a local cultivar.



Graph 3 Inhibitory activity of ethanolic extracts of Kodithotakalli leaves of *Piper betel* L. Cv. Kapoori, a local cultivar.



Graph 4 Inhibitory activity of methanolic extracts of Kodithotakalli leaves of *Piper betel* L. Cv. Kapoori, a local cultivar.

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DISCUSSION

The different solvent extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori tested were inhibitory to all the test organisms at various levels. The ethanol and ether extracts showed high activity against bacteria. The chloroform extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori showed increased zones of inhibition for the test bacteria Corynebacterium diphtheria and Pseudomonas aeruginosa. The methanol extract of Kodithotakalli leaves of Piper betel L. Cv. Kapoori were inhibitory to all the test organisms of which, Corynebacterium diphtheria was found to be highly sensitive. The ethanol extract also exhibited high activity against the growth of Corynebacterium diphtheria, in comparison with other organisms. Corynebacterium diphtheria was also found to be highly susceptible to the ether extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori, a local cultivar. Among the solvent extracts, ether extracts of Kodithotakalli leaves of Piper betel L. Cv. Kapoori, a local cultivar exhibited high antibacterial activity even at low concentrations. The inhibitory activity of the various solvent extracts was dose-dependent, enhanced with increase in concentration.

CONCLUSION

The secondary metabolites produced by plants mostly serve as plant defensive agents against microorganisms, insects and herbivores, in which pytocuratives play a vital role in treating various infectious diseases (S.H.K.R. Prasad, 2009). Researched plant parts in various species include the roots, seeds, latex, lactiferous tubes, stem wood, stem barks, leaves and whole plants. Many studies have suggested that plant species have therapeutic relevance (Hohman, 2004). Healing property of the Piper betel L. (Green gold of India) phenol, allylpyrocatechol against indomethacin-induced stomach ulceration and mechanism of action has been scientifically proved (Bhattacharya et al, 2007). The results of the present study suggest that the solvent extracts are effective against the tested organisms. The values of zone of inhibition reveal the medicinal properties of the experimental plant. Some extracts showed better activity than the standard. The results obtained in the present work may also provide a support to the use of the plant in traditional medicine. Further work is needed to isolate the active principle from the plant extracts and to carry out pharmaceutical studies.

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