Bio prospecting Mokkathotakalli Leaves of Piper Betel L. Cv. Kapoori as a Potential Source of Anti Microbial Agents against selected Bacterial Strains

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Abstract: ***India is a trove house of an ample variety of medicinal plants. Some species are observed wild, while a number of species have been domesticated by the farmers. India is known to be the richest archive of medicinal plants amongst the ancient civilizations, about 8,000 herbal remedies have been summarised in Ayurveda. The recorded medicinal plants in Rigveda (5000 BC) – 67species, Yajurveda 81 species, Atharvaveda(4500-2500 BC) 290 species, Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had detailed the properties and uses of 1100 and 1270 species respectively, in synthesizing of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine. It has been approximated that, in India, plant drugs enact as much as 80% of the total drugs. India is the world's 12th biodiversity centre with the ubiety of over 45000 different plant species. In India, medications of herbal origin have been used in customary medicines such as Unani and Ayurveda. Traditional systems of medicine keep on to be broadly practised on plentiful accounts. Many of the green plants synthesizes and put up a variety of biochemical products, many of which are extractable and employed as chemical feed stocks or as raw material for distinct scientific investigations. In accord with this information, Piper betel L. (Green gold of India), which is commonly considered as a traditional medicinal plant, was choosen for the study. Solvent extracts of Mokkathotakalli leaves of Piper betel L. Cv. Kapoori made 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. Among the solvent extracts, ether extracts of Mokkathotakalli of leaves of Piper betel L. Cv. Kapoori, exhibited leading antibacterial activity compared with other solvent extracts.***

Keywords: *Piper betel* L. Cv. Kapoori, Mokkathotakalli leaves, solvent extracts and antibacterial activity.

1. Introduction

The appliance of plants as remedy is as aged as human civilization itself. Many of the existing medicinal systems such as Ayurveda, Unani, Homeopathy, Naturopathy, Siddha and other alternative medicinal systems have been utilizing plants as effective source of medicines to cure many diseases. India being the largest producer of medicinal herbs is appropriately called the Botanical Garden of the World [1]. 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 and stimulant and for arthritis [2]. The betel leaf plant is a climbing slender stemmed branching vine as high as 10-15 feet [3]. The principal aim of the work was to study the antibacterial activity of Piper betel L. Cv. Kapoori Mokkathotakalli leaf extracts 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 Mokkathotakalli leaves of the Piper betel L. Cv. Kapoori, a local cultivar have been investigated.

2. Plant Material

2.1 Plant collection, identification and authentication

The Piper betel L. Cv. Kapoori (a local cultivar) Mokkathotakalli leaves 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/2011-002) was deposited at Department of Botany, Acharya Nagarjuna University, Nagarjuna nagar, Guntur, Andhra Pradesh, India for future

reference.

2.2. Extract Preparation

Fresh plant material was cleansed carefully under tap water, shade dried and used for extraction. The dried Mokkathotakalli leaves were homogenized to a fine powder and stored in airtight bottles. 25g of Mokkathotakalli leaves powder was extracted with 150 ml of solvent (chloroform, ethanol, ether and methanol) for 24h using Soxhlet apparatus. The extract then 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 which was used for extraction.

2.3. 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, 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 1×108 cfu/ml using UV Visible Spectrophotometer. By reading the OD of the solution to 0.45Å (610nm) it was used for further studies [4].

Table 1: List of the selected test organisms (bacteria)

S.No	Name of the organism	Characteristic 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)

2.4. Antibacterial Assay

Different concentrations of solvent extracts of the Mokkathotakalli leaves of Piper betel L. Cv. Kapoori (a local cultivar) were tested for antimicrobial activity by using Antibiotic Sensitivity test [5, 6]. Bacterial suspension was evenly mixed with sterile Agar medium and poured into the 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.

3. Results

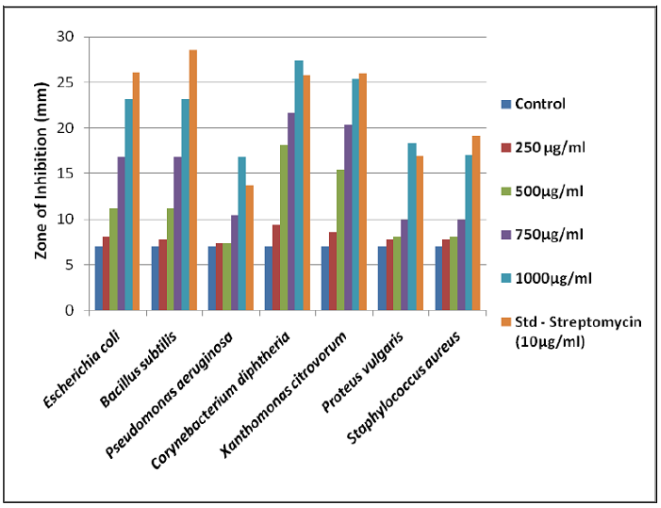
The results of the study are given in Table 2 and graphical representations of the each solvent given separately as Graph 1, 2, 3 and 4. The results indicate that the ether extracts of Mokkathotakalli leaves of Piper betel L. Cv. Kapoori were inhibitory to all the test organisms.

Table 2: Inhibitory activity of solvent extracts of Mokkathokalli 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	8.05	7.84	7.35	9.45	8.61	7.77	7.77
	500	11.20	11.20	7.35	18.20	15.40	8.05	8.05
	750	16.80	16.80	10.50	21.70	20.30	9.94	9.94
	1000	23.10	23.10	16.80	27.30	24.50	18.34	17.10
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	9.00	9.00	9.00	9.00
	750	11.80	11.80	15.30	13.90	13.90	13.20	13.20
	1000	15.30	15.30	18.80	18.80	18.10	18.49	17.70
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	10.40	10.40	9.00	10.40	9.00	9.70	9.84
	750	14.60	14.60	11.80	15.30	11.80	11.52	17.70
	1000	15.86	15.86	12.92	17.33	12.92	13.62	19.10
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	8.80	8.80	8.60	11.68	8.60	8.60	11.13
	750	10.98	10.98	8.88	14.48	8.88	11.40	17.63
	1000	13.78	13.78	11.68	19.38	12.38	11.40	29.18
Std*		26.03	28.55	13.73	25.81	26.00	17.00	19.08

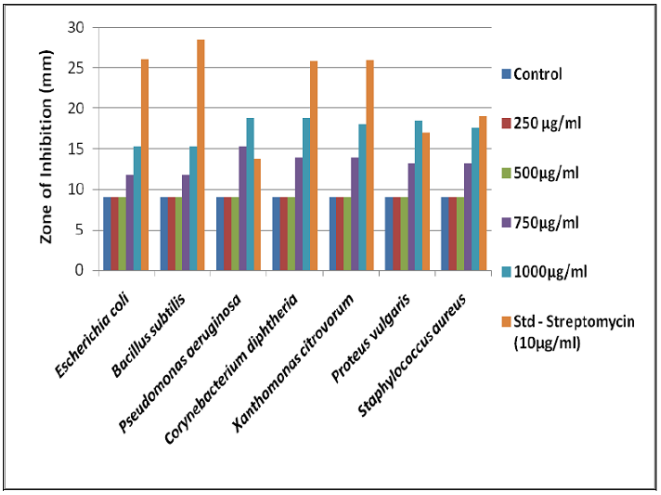
*Standard: Streptomycin (10µg/ml) for Bacteria

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 tested bacterial species, Corynebacterium diphtheria was found to be highly sensitive to the ether extracts of Mokkathotakalli leaves of Piper betel L. Cv. Kapoori, a local cultivar (Graph 1).



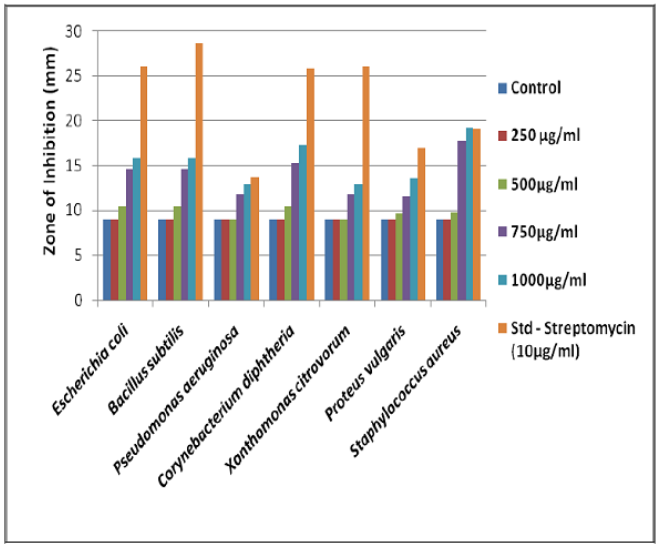
Graph 1: Inhibitory activity of ether extracts of Mokkathokalli leaves of Piper betel L. Cv. Kapoori, a local cultivar

The chloroform extracts of the Mokkathotakalli leaves of Piper betel L. Cv. Kapoori were also inhibitory to all the test organisms (Graph 2).



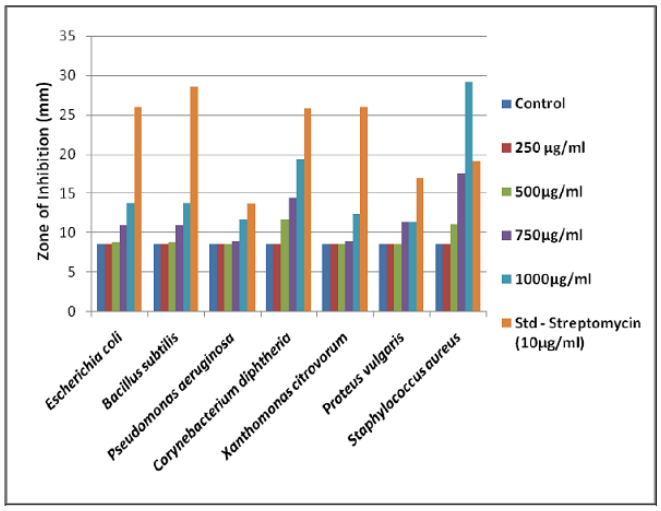
Graph 2: Inhibitory activity of Chloroform extracts of Mokkathokalli leaves of Piper betel L. Cv. Kapoori, a local cultivar

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 Mokkathotakalli 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 Mokkathotakalli 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 750µg/ml for Pseudomonas aeruginosa and Xanthomonas citrovorum. Of all the bacterial species tested, Corynebacterium diphtheria was seen to be highly susceptible to the ethanol extracts of Mokkathotakalli leaves of Piper betel L. Cv. Kapoori (Graph 3).



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

The methanol extracts of Mokkathotakalli leaves of Piper betel L. Cv. Kapoori also inhibited all the test organisms (Graph 4). The observed minimum inhibitory concentration was 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 Mokkathotakalli leaves of Piper betel L. Cv. Kapoori, a local cultivar.



Graph 4: Inhibitory activity of Methanolic extracts of Mokkathokalli leaves of Piper betel L. Cv. Kapoori, a local cultivar

4. Discussion

The different solvent extracts of Mokkathotakalli 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 Mokkathotakalli 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 Mokkathotakalli 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 illustrated high activity against the growth of Corynebacterium diphtheria, in comparison with other organisms. Corynebacterium diphtheria was also found to be very affected to the ether extracts of Mokkathotakalli leaves of Piper betel L. Cv. Kapoori, a local cultivar. Among the solvent extracts, ether extracts of Mokkathotakalli 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.

5. Conclusion

The secondary metabolites produced by plants essentially are of use as plant defensive agents against microorganisms, insects and herbivores, in which phytocuratives play a vital role in treating various infectious diseases [7]. Probed plant parts in various species include the roots, seeds, latex, lactiferous tubes, stem wood, stem barks, leaves and whole plants [8]. Many studies have suggested that plant species have therapeutic relevance [9]. 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 [10]. Methanolic extracts of the leaves of Terminalia catappa L., Manilkara zapota L., Piper betel L. were showed more efficacy against 10 Gram +ve, 12 Gram –ve bacteria and 1 fungal strain; Among the three plants, the most active antimicrobial plant was Piper betel L.[11]. The results of the present study suggest that the solvent extracts are effective against the tested microorganisms. The values of zone of inhibition reveal the medicinal properties of the experimental plant. Some extracts showed better activity than the standard. The results thus acquired in the present work may also provide a support to the use of the plant in traditional medicine. Additional work is essential to isolate the active principle from the plant extracts and to carry out pharmaceutical studies.

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References

[1] Ahmedulla M, Nayer M.P. Red data book of Indian plants. Volume- 4.Calcutta: Botanical survey of India, 1999.

[2] Kirtikar, K.R, Basu, B.D. Indian Medicinal Plants, 2nd ed.; Bishen Singh Mahendra Pal Singh: Dehradun, India,; Vol. III, pp. 2131-2133, 1998.

[3] Prajapathi, N. D., Agro's Dictionary of Medicinal Plants, Agrobios, Jodhpur, p. 401, 2003.

[4] K. Anthonamma, S.H.K.R.Prasad, D. Rajasekhar, N.L. Swapna and Madan Prasad. In vitro antimicrobial efficacy of solvent extracts of seeds of Albizzia lebbeck (L.) Benth. International Journal of Advances in Pharmaceutical Sciences, 1, 281-283, http://www.arjournals.org/ijoaps.html, 2010.

[5] Barry, A.C. Standard diffusion methods for antibiotic susceptibility of common rapid growing bacterial pathogens, Baltimore, USA: Park press, 1976.

[6] Benson, H.J. Microbiological applications, 5th ed. USA: WMC. Brown Publ,34, 1990.

[7] S. H. K. R. Prasad, N. L. Swapna, D. Rajasekhar, K. Anthonamma and Madan Prasad, Preliminary Phytochemical and Antimicrobial Spectrum of Cultured Tissues of Physalis minima (L.), Int. J. Chem. Sci.: 7(4), 2719-2725, 2009.

[8] Parekh J, Jadeja D, Chanda S. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turk J Biol, 29, 203-210, 2005

[9] Hohmann J, Molnár J. Plant toxins are promising molecules for the therapy, Acta Pharm. Hung, 74, 149–157, 2004.

[10] Bhattacharya S, Banerjee D, Bauri A K, Chattopadhyay S, Bandyopadhyay S K . Healing property of the Piper betel phenol, allylpyrocatechol against indomethacin-induced stomach ulceration and mechanism of action, World J Gastroenterol, 13(27): 3705-3713, 2007.

[11] N. L. Swapna, K Ammani and Harikrishna.R.P. Saripalli, Antibacterial efficacy of Mokkathotapapada leaf extracts of Piper betel L. Cv. Kapoori, a local cultivar (Green gold of India), Journal of Chemical, Biological and Physical Sciences; An International Peer Review E-3 Journal of Sciences, J. Chem. Bio. Phy. Sci. Sec. B, Vol.3, No.1, 201-209, 2012-2013.

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