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Evaluation of Antimicrobial Activity of Leaf Extracts Of *Pimpinella tirupatiensis.*

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ABSTRACT

Pimpenella tirupatensis is an endemic medicinal plant of Andhra Pradesh and is known for its cardio protective, hepatoprotective, antihyperglycemic and antihyperlipidamic activity. The antimicrobial activity of different concentrations (50 to 150 µg/mL) of *p.tirupatensis* leaf extracts in various solvents (Acetone, methanol, ethyl acetate, chloroform, and hexane) was evaluated against pathogenic bacterial strains (*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhimurium, Proteus mirabilis, Bacillus subtilis, and Proteus vulgaris).* Among all the extracts, tested leaf acetone extract displayed strong antimicrobial activity against *Proteus mirabilis (*inhibition zone diameter 16 mm). It also exhibited low values of both MIC and MBC.

Keywords: *Pimpenella tirupatensis,* Antimicrobial activity, Inhibition zone diameter MIC, MBC.

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INTRODUCTION

Plants and plant bio active compounds have been a source of medicine in the past centuries. Even today, scientists and the general public are familiar with their value as a source of new and complimentary medicines owing to their adaptable applications [1]. Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compounds as antimicrobial agents. Medicinal plants are the richest bio resources of drugs for traditional medicinal systems, current medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals and intermediate chemicals [2, 3].

The substances that can inhibit pathogens and have little toxicity to host cells are considered to be excellent candidates for developing new antimicrobial drugs [4].

When scientific articles are compared, the differences between the plant derived constituents and their concentrations can result from several factors, such as intraspecific genetic variability, environmental aspects, collection times, growing conditions, soil type, and part of the plant analyzed, which can influence both the content of compounds present in the essential oil and its chemical composition[5].

p.tirupathansis belonging to family Apiaceae is distributed in the forest of Tirupati in Andhra Pradesh,india commonly known as adavi kothimeera (Forest Coriander). It is used for the treatment of External inflammation, Diuretic, treatment of bladder distress, Asthma, Aphrodisiac, Skin diseases, Ulcers, Blood disorders, Toothache and Hepatoprotective [6].

The ethanolic extracts of *p.tirupatiensis* exhibited different degrees of inhibitory activity against (*Micrococcus luteus, Micrococcus roseus, Pseudomonas aeruginosa, Staphylococcus aureus* (bacterial strains) and *Candida albicans* (fungal strain)) human pathogenic microorganisms at different concentrations [7].

The tuberous roots of *p. tirupatiensis* hexane *and* ethyl acetate extracts were tested against eight bacterial and three fungal pathogenic strains for antimicrobial activity [8].

Plants as new sources of antimicrobial agents have many advantages viz, increasing awareness of drug resistance of antibiotics owing to their over use. Further a multitude of phytopharmaceuticals are readily available and display excellent antimicrobial activity without any toxicity to human being. “Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug” [9].

As the plant species are proceeding towards extinction a sense of urgency is required plants have to be investigated a new for their antimicrobial usefulness.

MATERIALS AND METHODS

Plant materials

The Plant material of *p.tirupatiensis* used for present investigation was collected from Seshachalam forest of Tirupati & identification has been done by Prof. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India (voucher no: 1208).

Extraction of plant material

The leaves of the *p.tirupatensis* were dried in the shade at room temperature and ground to powder. Fifty grams of powdered plant material was extracted in 200ml of each solvent (Acetone, Ethyl Acetate, Chloroform, Methanol and Hexane) separately and kept on orbital shaker for 48 hrs. The extracts were filtered through whatmann filter paper after 48hrs and concentrated using rota evaporator under reduced pressure to yield the residue. These extracts were further used to evaluate their antimicrobial activity

Determination of antimicrobial activity

Test organisms

The test organisms used in this study were combination of gram +ve and gram –ve pathogenic bacteria. *Escherichia coli* (MTCC 7410)*, Staphylococcus aureus* (MTCC 7443)*, Salmonella typhimurium* (MTCC 98)*, Bacillus subtilis* (MTCC 511)*, Klebsiella pneumonia* (MTCC 3384)*, Proteus mirabilis* (MTCC 425)*, Proteus vulgaris* (MTCC 744)*,* and *Pseudomonas aeruginosa* (MTCC 2295) were procured from IMTECH, Chandigarh. The bacterial cultures were maintained on Mueller-Hinton agar slants at 4˚C with a subculture period of 15 days. Each bacterial strain was reactivated from the stored slants to Mueller-Hinton broth and cultured overnight at 37˚C before the antimicrobial assay **[10].**

Antibacterial susceptibility assay

The antimicrobial activities of extracts were determined by two methods including disc diffusion test and broth dilution assay [10].

Sterile disc of 5 mm diameter was saturated with 20 µl of the different concentrations of extract solution ranging from 50 to 150 (µg/mL). The paper discs were dried and placed on the surface of the inoculated agar plates. Plates were kept for 1 h in refrigerator to enable prediffusion of the extracts into the agar. Then the inoculated plates with test microorganisms were incubated at 37^0C for overnight to allow bacterial growth.

Amikacin was used as positive control. The antibacterial activities of the extracts were evaluated by measuring the inhibition zones.

Determination of MIC and MBC

The MIC and MBC of various leaf extracts was determined by broth micro-dilution method **[11]** and modified according to the lab conditions.

Statistical analysis

The Inhibition zone diameter of five extracts of different concentrations were determined by linear regression analysis method, results were expressed as mean ± SD (standard deviation).

RESULT AND DISCUSSION

The Antimicrobial activities of various leaf extracts in different concentrations ranging from 50 to 150 µg/mL were evaluated against different human pathogens. The activity was recorded as inhibition zone diameter (IZD).

The acetone extracts of *p.tirupatensis* displayed promising antimicrobial activity against all the pathogens compared to the rest of the extracts (table 1). The acetone extracts showed highest activity against bacterial strains, *Pseudomonas aeruginosa, Proteus vulgaris ,Salmonella typhimurium, Escherichia coli ,Klebsiella pneumoniae ,Staphylococcus aureas,Bacillus subtilis, and Proteus mirabilis* at (150 µg/mL) concentration with zone diameter 11mm, 13mm, 11mm, 16mm, 15mm,14mm,12mm and,13mm respectively. The antimicrobial activity of all the extracts was found to be concentration dependent. Hexane extracts at higher concentration (150µg/mL) exhibited antimicrobial activity against *E.coli, Proteus mirabilis, and Salmonella typhimurium* with zone of diameter *10* mm, 8 mm and 9 mm respectively

The ethyl acetate showed inhibitory effect against some bacterial strains *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa* 10 mm, 11mm, and 11 mm respectively. The chloroform and methanol extracts also showed activity low compare to acetone extracts and high compare to hexane and ethyl acetate against all tested pathogens.

MIC and MBC of various leaf extracts of *p.tirupatensis* have been displayed in (Table 2). The minimum MIC value was observed for leaf acetone extract against *Staphylococcus aureas* which is comparable standard antibiotic.

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Table1: Antimicrobial activity of *p.tirupatensis* leaf extracts (µg/mL).

Extract	Test Organisms	Inhibition zone diameter (mm)			
		50	100	150	Amikacin
Leaf- Acetone	*Pseudomonas aeruginosa*	8.±0.20	9±0.30	11±0.33	23±0.40
	Proteus vulgaris	6.±0.20	9±0.30	13±0.41	24±0.26
	Salmonella typhi	7±0.75	9±0.60	11±0.41	23±0.43
	Proteus mirabilis	6±0.36	12±0.43	16±0.34	24±0.416
	Klebsiellapneumoniae	10±0.20	11±0.34	15±0.48	26±0.473
	Staphylococcus aureas	7±0.34	9±0.30	14.±0.47	24±0.45
	Bacillus subtilis	6±0.41	12±0.32	13±0.37	25±0.153
	Escherichia coli	6±0.20	9±0.20	12±0.35	24±0.49
Leaf -Methanol	*Pseudomonas aeruginosa*	6±0.57	8±0.54	9±0.57	23±0.40
	Proteus vulgaris	6±0.53	7±0.67	8±0.57	24±0.26
	Salmonella typhi	6±0.54	6±0.57	6±0.57	23±0.43
	Proteus mirabilis	-	-	8±0.57	24±0.416
	Klebsiella pneumoniae	-	8±0.57	11±1.0	26±0.473
	Staphylococcus aureas	-	-	9±0.57	24±0.45
	Bacillus subtilis	-	-	8.4±0.53	25±0.153
	Escherichia coli	6±0.50	8±0.20	9 ±0.43	24±0.49
Leaf -Hexane	*Pseudomonas aeruginosa*	-	-	-	23±0.40
	Proteus vulgaris	-	-	-	24±0.26
	Salmonella typhi	-		8±0.55	23±0.43
	Proteus mirabilis	-		9 ±0.64	24±0.416
	Klebsiellapneumoniae	-	-	-	26±0.473
	Staphylococcus aureas	-	-	-	24±0.45
	Bacillus subtilis	-	-	-	25±0.153
	Escherichia coli	-		10±0.60	24±0.49
Leaf -Ethyl acetate	*Pseudomonas aeruginosa*	-	6±0.20	11±0.37	23±0.40
	Proteus vulgaris	-	-	-	24±0.26
	Salmonella typhi	-	-	-	23±0.43
	Proteus mirabilis	-	-	11±0.60	24±0.416
	Klebsiellapneumoniae	-	-	-	26±0.473
	Staphylococcus aureas	-	-	-	24±0.45
	Bacillus subtilis	6±0.11	7±0.26	10±0.36	25±0.153
	Escherichia coli	-	-	-	24±0.49
Leaf- Chloroform	*Pseudomonas aeruginosa*	-	9±0.37	-	23±0.40
	Proteus vulgaris	-	-	-	24±0.26
	Salmonella typhi	-	-	-	23±0.43
	Proteus mirabilis	-	-	11±0.60	24±0.416
	Klebsiellapneumoniae	-	-	-	26±0.473
	Staphylococcus aureas	-	-	-	24±0.45
	Bacillus subtilis	-	8±0.36	-	25±0.153
	Escherichia coli	-	-	-	24±0.49

The values are of mean ± SD (n=3) of three replicates.

Our results indicate that acetone yielded more potent extract with higher antimicrobial activity thus inhibiting the highest number of bacterial strains. This may also be attributed to the presence of soluble phenolic and polyphenolic compounds [12]. The results are also in confirmation with some recent studies on *Rumex dentatus* [13, 14].The lack of antibacterial activity in some of the concentrations of the extract is not surprising as a number of plant extracts which have been found ineffective against certain test organisms at lower concentrations may work better at higher concentration owing to the higher concentration of antimicrobial phytochemical [15]. The antibacterial effects of the extracts could be explained by disturbance of the permeability barrier of the bacterial membrane structure [16].

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Table 2: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *p.tirupatensis* leaf extracts (µg/mL) against test organisms.

Test organisms	Acetone		Methanol		Hexane		Ethyl acetate		Chloroform		Amikacin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	125	250	125	250	500	1000	125	250	500	1000	7.8	15.6
Salmonella typhi	125	250	250	125	500	1000	125	250	250	500	15.6	31.2
Proteus vulgaris	62.5	125	125	250	500	1000	500	1000	500	1000	7.8	15.6
Proteus mirabilis	250	125	250	500	500	1000	125	250	500	1000	7.8	15.6
Pseudomonas aeruginosa	250	500	125	250	500	1000	125	250	500	1000	7.8	15.6
Klebsiella pneumoniae	125	250	125	250	500	1000	250	500	250	500	7.8	15.6
Staphylococcus aureus	31.2	62.5	125	250	250	500	62.5	125	500	1000	3.9	7.8
Bacillus subtilis	125	62.5	125	250	500	1000	500	1000	250	500	7.8	15.6

It was suggested that extract components cross the cell membrane, interacting with enzymes and proteins of the membrane, thus producing a flux of protons towards the cell exterior which induces changes in the cells and, ultimately their death [17]. It is evident from the results of the current study that susceptibility of pathogens to plant extracts depends upon solvent used for extraction, extract concentration and the organism tested which is in corroboration with the studies of [18, 19, 20].

Even though the recent interest in drug discovery using molecular modelling, combinatorial chemistry, and other synthetic chemistry methods, natural product-derived compounds are still proving to be an invaluable source of medicines for humans [21].

The antibacterial activity of *p.tirupatensis* was evaluated previously with only some solvents and tested against a few bacterial strains [7, 8]. However, not all of them were tested against the strains at different concentrations used in this study. Interestingly, no previous study has reported the antibacterial activity of *p.tirupatensis* leaf extracts in various solvents (Acetone, methanol, ethyl acetate, chloroform, and hexane), which showed a good activity against the Gram- negative with the MIC values in the range 50–500 µg/mL.

Antimicrobial plant extracts have an important place in clinical microbiology. Theses phytochemicals may find their way into the antimicrobial drug arsenal used by physicians. These are efforts to search for new anti-infective agents as the effective lifespan of an antibiotics limited.

After identification of antimicrobial agents from plants a more systemic study is required to evaluate their effectiveness in whole organism, level which includes toxicity and effects on beneficial microbiota.

REFERENCES

[1] Chaudhary G, Goyal S, Poonia P. Lawsonia inermis Linnaeus: Int J Pharm Sci Drug Res 2010; 2(2): 91-98.
[2] Das K, Tiwari RKS, Shrivastava DK. J Med Plant Res 2010; 4(2): 104-111.
[3] Anwer N, Waqar MA, Iqbal M, Mushtaq M, Sobia A. Int Food Res J 2013; 20(1): 99-103.
[4] Bajpai M, Pande A, Tewari SK, Prakash D. International Journal of Food Sciences and Nutrition 2005; 56(4): 287–291.
[5] Scur MC, et al. Brazilian Journal of Biology 2016; 76(1): 101-108.
[6] Kirtikar KR, Basu BD. International book distributors, 1987; 3(2):2061- 2062.
[7] Jeevan Ram A, Md. Bhakshu L, Venkata Raju RR. J Ethnopharmacol 2004; 90: 353-357.
[8] Bakshu, L. M., & Venkata Raju, R. R. Flavour and fragrance Journal 2002, 17(6): 413-415.
[9] Cowan MM. Clinical Microbiology Reviews. 1999; 12(4):564-82.
[10] Kim J, Marshall MR, Wie C. Journal of Agricultural and Food Chemistry 1995; 43:2839–2845.
[11] Alade,PI.,& Irobi,O.N. J Ethnopharmacol 1993; 39: 171–174.
[12] Perez A, Orozco M, Rivas V, Waksman N. Nat Prod Commun 2008; 3: 363- 368.
[13] Lone BA, Bandh SA, Chishti MZ, Bhat FA, Tak H, Nisa H. Trop Anim Health Pro, 2012; 45: 743-749.
[14] Bandh SA, Kamili AN, Ganai BA, Lone BA, Saleem S. J Pharm Res 2011; 4(9): 3141-3142.

[15] Amal A, Ashraf A, Hossam ES. Journal of Biyoloji Bilimleri Arastirma Dergisi 2009; 2(2): 71–79.
[16] Hayek, S. A., & Ibrahim, S. A. International journal of microbiology, 2012.
[17] Moghadam MS, Maleki S, Darabpour E, Motamedi H, MansourS, Nejad S. Asian Pac J Trop Med 2010;
[18] 3: 262- 265.
[19] Chaudhary G, Goyal S, Poonia P. Lawsonia inermis Linnaeus: Int J Pharm Sci Drug Res 2010; 2(2): 91-98.
[20] Usman H, Abdulrahman FI, Usman A. AfrJ Tradit Complement Altern Med 2009; 6(3): 289- 295.
[21] Hadizadeh I, Pivastegan B, Hamzehzarghani H. Am J Appl Sci 2009; 6: 857-861.
[22] Vu TT, et al. BMC Complementary and alternative medicine, 2016, 16(1):1