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Research Article

ANTIBACTERIAL POTENTIAL OF TRADITIONAL PLANT SPECIES *SOLENA AMPLEXICAULIS* (LAM.) GANDHI. AGAINST CERTAIN HUMAN PATHOGENS

K. KARTHIKA AND S. PAULSAMY*

Department of Botany, Kongunadu Arts and Science College, Coimbatore – 641 029; Email: paulsami@yahoo.com

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ABSTRACT

Solena amplexicaulis (Cucurbitaceae) is a traditional medicinal climber generally prescribed for wound healing by the local healers in western districts of Tamil Nadu. The study was conducted with the objective to evaluate the antibacterial activity of the aqueous and alcoholic extracts of leaf and stem parts of this species against 15 human pathogenic bacteria by adapting disc diffusion method. The results of the study revealed that all extracts showed varied degree of antibacterial activity against the tested pathogens. However, the methanolic leaf and stem extracts exhibited higher inhibition zone (16mm and 23mm respectively) against the bacterium, *Bacillus subtilis.* Minimum inhibitory concentrations (MIC) exhibited by methanolic leaf and stem extracts against the tested organisms were ranging between 200 to 400µg/mL and 300 to 500µg/mL respectively. The results of the study support the therapeutic importance of the species, *S. amplexicaulis* in curing infectious diseases and so it can be encouraged for its extensive use in health care practices.

Keywords: *Solena amplexicaulis,* antibacterial activity, disc diffusion method, Minimum inhibitory concentration.

INTRODUCTION

Plants have been important source of medicine for thousands of years. They are capable of synthesizing a diverse array of secondary metabolites[1] which are the basis for the discovery and development of new drugs of natural origin. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases[2]. There are several reports on the antimicrobial activity of different herbal extracts against many human pathogens[3,4,5]. The plant species with more promised antimicrobial activities are identified as potent source for the anti-infective drug development[6].

Solena amplexicaulis is a perennial dioecious climber with tuberous root found throughout Asia mainly growing in hilly dry deciduous forests, scrub jungles. The tubers, leaves and seeds are extensively used in traditional system for various ailments like hepatosplenomegaly, spermatorrhoea, appetizer, cardiotonic, diuretic and thermogenic[7]. Root is stimulant and purgative[8]. The leaves have good anti-inflammatory activity and also prescribed for skin lesions and other skin diseases[9]. The whole plant is determined to be a potential source of natural antioxidant activity[10,11] and also used for the treatment of diabetes[12].

The present study aimed at to evaluate antibacterial activity of different alcoholic and aqueous extracts of leaf and stem parts of *S. amplexicaulis* and to determine minimum inhibitory concentration (MIC) of appropriate alcoholic extract of these parts to be determined against certain both Gram positive and Gram negative bacterial human pathogens.

MATERIALS AND METHODS

Plant material

The leaf and stem parts of *S.amplexicaulis* were collected from a scrub jungle in Madukkarai, Coimbatore district, Tamil Nadu, India. Collected plant materials were washed thoroughly in tap water, shade dried and then homogenized to fine powder and stored in air tight bottles.

Preparation of extracts

About 50g of powdered plant materials (50g/250ml) were extracted in a soxhelt extractor for 8 to 10 hours, sequentially with the alcoholic solvents *viz.,* hexane, benzene, chloroform and methanol, and water. Then the extracts were evaporated to dryness.

Bacterial strains

In vitro antibacterial activity was examined for the crude extracts of leaf and stem parts of the study plant against 15 bacterial species which include the Gram positive strains *viz.*, *Streptococcus faecalis, S. pyogenes, Bacillus subtilis, B. thuringiensis, Staphylococcus aureus* and *Enterococcus faecalis* and Gram negative strains *viz., Klebsiella pneumoniae, Salmonella paratyphi, S.paratyphi* A, *S. paratyphi* B, *Escherichia coli, Proteus vulgaris, P. mirabilis, Serratia marcescens* and *Pseudomonas aeruginosa.* All these bacterial strains were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore and they were maintained at 4°C on nutrient agar slants for further use.

Antibacterial assay

An inoculum of each of the pathogenic bacterial strains was suspended in 5ml nutrient broth and incubated at 37°C for 18 hrs. The antibacterial activity was tested by the disc diffusion assay[13]. For this, the inoculum was spread over Muller-Hinton agar medium with sterile glass spreader. Small circular paper discs (5mm diameter) impregnated with known amount of each extract was placed upon the surface of the inoculated plates. The plates were kept at room temperature for absorption of extract in the medium and then incubated at 37°C in the incubator for 24 hrs. The antibacterial activity was evaluated by measuring the diameter of inhibition zone[14]. Ampicillin was used as positive control. Triplicates were maintained for all experiments.

Minimum inhibitory concentration (MIC)

For the determination of MIC, 10 test tubes each with 1800µl of nutrient broth were taken for each bacterial strain. Different concentrations of leaf and stem extracts ranged from 100 to 800µg/mL were incorporated into the broth and the tubes were then inoculated with 200µl of inoculum of respective bacteria (10^5 CFU/mL) and kept at 37°C for 24hrs. The test tube containing the lowest concentration of extract which showed reduction in turbidity, when compared with positive control (ampicillin) and negative control (solvent) was regarded as MIC of that extract[15].

RESULTS AND DISCUSSION

The data of antibacterial activity of aqueous and alcoholic solvent extracts (hexane, benzene, chloroform and methanol) of leaf and stem parts of the study species, *Solena amplexicaulis* against 15 human pathogenic bacteria on basis of the magnitude of zone of inhibition are presented in Tables 1 and 2. It showed that the zone of inhibition ≥ 9-15mm is an indication of the existence of strong antimicrobial activity in these parts[16]. Among the five extracts attempted, the methanolic extracts of both parts showed highly significant activity. On the other hand, the hexane extracts showed lesser activity against both Gram +ve and Gram –ve bacteria. Further, all the bacteria were found to be most susceptible to methanolic leaf extract and among them, the maximum zone of inhibition has been produced against the bacteria, B*acillus thuringiensis* (17±2.1mm), *B. subtilis* (16±2.0mm) and *Proteus*

vulgaris (15±0.3mm) and less inhibition was observed on *Streptococcus pyogenes* (8±1.0mm). The bacterial strain, *Serratia marcescens* was found to be resistant to all extracts excepting to the methanolic leaf extract (10±1.7mm). For the stem extract, the maximum zone of inhibition was observed for the bacterial strains *Bacillus subtilis* (23±2.5mm), *Staphylococcus aureus* (15±1.1mm) and *Klebsiella pneumoniae* (15±2.0mm) which was significantly higher than the zone of inhibition caused by the standard drug, amphicillin. However, it showed less inhibitory activity against the bacterium, *Salmonella paratyphi* B (8±1.3mm).

Table 1: Antibacterial activity of the leaf extracts of *Solena amplexicaulis* on certain pathogenic bacteria.

Plant extracts	Diameter of inhibition zone (mm)														
	Gram positive bacteria							Gram negative bacteria							
	SF	SP1	BS	BT	SA	EF	KP	SP2	SP3	SP4	EC	PV	PM	SM	PA
Control*	15±1.8[a]	15±0.6[a]	21±2.3[a]	15±0.7[a]	18±1.3[a]	9±0.8[a]	15±1.8[a]	20±1.8[a]	30±1.5[a]	8±1.3[a]	14±0.3[a]	15±1.1[a]	26±1.2[a]	10±0.6[a]	11±1.1[a]
Hexane	-	-	-	-	-	-	-	-	-	6±1.5[b]	-	-	-	-	-
Benzene	7±0.6[b]	-	8±1.9[b]	-	-	6±1.1[b]	-	7±0.8[b]	6±0.9[b]	10±0.7[c]	7±0.6[b]	-	9±0.7[b]	-	7±1.3[b]
Chloroform	8±2.0[b]	-	8±1.9[b]	-	-	6±0.2[b]	-	-	7±0.5[b]	11±0.9[cd]	7±0.9[b]	-	-	-	-
Methanol	11±1.8[c]	8±1.0[b]	16±2.0[c]	17±2.1[a]	11±1.2[b]	10±0.8[a]	14±1.5[a]	12±0.9[c]	12±0.8[c]	13±1.3[d]	11±1.2[c]	15±0.3[a]	14±1.1[a]	10±1.7[a]	13±1.5[a]
Water	8±1.3[b]	6±0.4[b]	8±1.5[b]	7±1.6[c]	9±0.8[b]	-	7±1.7[b]	-	8±0.5[b]	-	-	8±1.2[b]	8±1.3[b]	-	-

Values are expressed as mean ± SD of three parallel measurements.
Means followed by different letter(s) in columns are significant to each other at 5% level according to DMRT.
Ampicillin, SF-*Streptococcus faecalis,* SP1 – *S. pyogenes,* BS - *Bacillus subtilis,* BT - *B. thuringiensis,* SA - *Staphylococcus aureus,* EF - *Enterococcus faecalis* KP - *Klebsiella pneumoniae,*SP2 - *Salmonella paratyphi,* SP3 - *S. paratyphi* A, SP4 - *S. paratyphi* B, EC - *Escherichia coli,* PV - *Proteus vulgaris,* PM - *P. mirabilis,* SM - *Serratia marcescens* and PA - *Pseudomonas aeruginosa.

Table 2: Antibacterial activity of the stem extracts of *Solena amplexicaulis* on certain pathogenic bacteria.

Plant extracts	Diameter of inhibition zone (mm)														
	Gram positive bacteria							Gram negative bacteria							
	SF	SP1	BS	BT	SA	EF	KP	SP2	SP3	SP4	EC	PV	PM	SM	PA
Control*	19±0.6[a]	15±2.0[a]	22±2.3[a]	12±0.6[a]	8±1.5[a]	11±0.3[a]	9±1.7[a]	20±0.3[a]	28±1.2[a]	12±1.0[a]	14±0.8[a]	10±0.5[ac]	26±0.9[a]	10±0.9[a]	12±1.1[a]
Hexane	-	-	13±1.8[bc]	-	-	-	-	-	7±0.3[b]	-	-	-	6±0.3[b]	-	-
Benzene	7±1.4[b]	-	15±1.5[b]	7±0.4[b]	7±0.9[a]	8±1.5[b]	8±0.8[a]	8±0.5[b]	10±0.5[c]	-	-	-	10±0.5[c]	6±1.5[b]	-
Chloroform	7±1.8[b]	7±1.2[b]	10±1.0[c]	7±0.2[b]	8±1.0[a]	7±0.8[b]	8±0.9[a]	7±0.4[b]	12±0.7[c]	8±1.1[b]	7±0.6[b]	8±0.8[b]	10±0.6[c]	6±1.8[b]	6±1.7[b]
Methanol	9±2.1[b]	10±1.5[c]	23±2.5[a]	10±0.3[a]	15±1.1[b]	13±1.3[a]	15±2.0[b]	10±1.2[c]	12±1.2[c]	8±1.3[b]	12±1.8[a]	12±0.9[c]	15±1.3[d]	11±0.5[a]	10±1.5[a]
Water	8±2.3[b]	8±1.8[b]	7±0.8[d]	8±0.1[b]	7±1.6[a]	10±1.3[a]	7±1.8[a]	8±1.1[b]	7±1.5[b]	-	-	-	8±0.8[e]	-	7±1.8[b]

Values are expressed as mean ± SD of three parallel measurements.
Means followed by different letter(s) in columns are significant to each other at 5% level according to DMRT.
Ampicillin, SF-*Streptococcus faecalis,* SP1 – *S. pyogenes,* BS - *Bacillus subtilis,* BT - *B. thuringiensis,* SA - *Staphylococcus aureus,* EF - *Enterococcus faecalis* KP - *Klebsiella pneumoniae,* SP2 - *Salmonella paratyphi,* SP3 - *S. paratyphi* A, SP4 - *S. paratyphi* B, EC – *Escherichia coli,* PV - *Proteus vulgaris,* PM - *P. mirabilis,* SM - *Serratia marcescens* and PA - *Pseudomonas aeruginosa.

Different solvents have been reported to have different capacity to extract phytoconstituents according to their solubility or polarity[2]. In the present study, the methanol solvent due to high polarity extracted high amount of chemical ingredients which in turn resulted maximum zone of inhibition. Further, it is explained that the broad spectrum of antibiotics in plants affect a wide range of bacteria which include both Gram positive and Gram negative types by targeting bacterial cell wall or cell membrane or interfere with essential bacterial enzymes like quinolones and sulfonamides and targeting bacterial protein synthesis[17,18]. Many studies are supporting that methanol extracts of several medicinal plant species are having higher antibacterial activities than that of any other alcoholic solvents[19,20,21]. High antibacterial effects in organic extracts for certain Cucurbitaceae members were already well documented (*Trichosanthes cucumerine*[22], *Citrullus colocynthis*[23] and *Coccinia grandis*[24]). Among the methanolic extracts of two parts studied, the leaf extract was determined to be more effective. It may be explained due to presence of rich variety of appropriate secondary metabolites that can interfere the growth of bacteria. The findings of this study show that methanolic crude extracts of both leaf and stem parts exhibited a considerable higher antibacterial activity.

Table 3: Minimum inhibitory concentration (MIC) of methanolic extracts of leaf and stem parts of *Solena amplexicaulis* on certain pathogenic bacteria.

Plant parts	Minimum inhibitory concentrations (µg/mL)														
	Gram positive bacteria							Gram negative bacteria							
	SF	SP1	BS	BT	SA	EF	KP	SP2	SP3	SP4	EC	PV	PM	SM	PA
Leaf	400	400	300	400	300	400	500	400	400	400	300	300	400	400	500
Stem	400	400	400	500	500	500	400	400	600	400	500	500	600	400	500

SF-*Streptococcus faecalis,* SP1 – *S. pyogenes,* BS - *Bacillus subtilis,* BT - *B. thuringiensis,* SA - *Staphylococcus aureus,* EF - *Enterococcus faecalis,* KP - *Klebsiella pneumoniae,* SP2 - *Salmonella paratyphi,* SP3 - *S. paratyphi* A, SP4 - *S. paratyphi* B, EC - *Escherichia coli,* PV - *Proteus vulgaris,* PM - *P. mirabilis,* SM - *Serratia marcescens* and PA - *Pseudomonas aeruginosa.*

Due to antibacterial activity of methanolic leaf and stem extracts, minimum inhibitory concentration (MIC) was determined only for methanolic extracts (Table 3). Available information through literature showed that MIC values between 50-500µg/mL exhibit strong activity, 600-1500µg/mL exhibit moderate activity and above 1500µg/mL exhibit weak activity[15]. Based on this fact, the present investigation confirms the leaf and stem extracts of *S. amplexicaulis* showed strong activity against all bacteria including both Gram +ve and Gram –ve, and the methanolic leaf extract showed strongest antibacterial activity against the bacterial strains, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* as the MIC values against these bacteria obtained were 300µg/mL.

CONCLUSION

The present research is a right step to the direction of searching for novel and more effective antibacterial compounds in plants. In conclusion the species, *S. amplexicaulis* extracts exhibited antibacterial activity against both Gram +ve and Gram –ve bacterial strains mediating the presence of a broad spectrum of antibacterial compounds. Further studies may be necessary to elucidate the specific phytoactive compounds in the leaf and stem extracts of *S. amplexicaulis* and hence to go for commercial application through pharmaceutical industries.

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REFERENCES

1. Wittstock U, Gershenzon J. Constitutive plant toxins and their role in defense against herbivores and pathogens. Current Opinion in Plant Biology 2002; 5(4): 300-307.
2. Marjorie MC. Plant product as antimicrobial agents. Clinical Microbiology Reviews 1999; 12: 564-582.
3. Chung PY, Chung LY, Ngeow YF Goh SH, Imiyabir Z. Antimicrobial activities of Malaysian plant species. Pharmaceutical Biology 2004; 42: 292-300.
4. Nair R, Chanda SV. Antibacterial activity of some medicinal plants of Saurashtra region. Journal of cell and tissue research 2004; 4: 117-120.
5. De N Ifeoma E. Antimicrobial effects of components of the bark extracts of Neem (*Azadirachta indica* A. Juss). Technology and Development 2002; 8: 23-28.
6. Edeoga HO, Okwu DE and Mbacbie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4: 685-688.
7. Kritchevsky D. Fiber, lipids and atherosclerosis. American Journal of Clinical Nutrition 1978; 31S: 65-74.
8. Goldin Quadros. Report of the 'Study of Biodiversity of Indian Institute of Technology, Bombay Campus' by World Wide Fund for Nature-India, Maharashtra State Office, Mumbai, India.
9. Arun Ch, Satheesh Kumar R, Srinu S, Lal Babu G, Raghavendra Kumar G Amos Babu J. Anti inflammatory activity of aqueous extract of leaves of *Solena amplexicaulis.* International journal of research in pharmaceutical and biomedical sciences 2011; 2(4): 1617-1619.
10. Venkateshwarlu E, Raghuram Reddy A, Goverdhan P, Swapna Rani K, Jayapal Reddy G. *In vitro* and *in vivo* antioxidant activity of methanolic extract of *Solena amplexicaulis* (whole plant). International Journal of pharmacy and biological science 2011; 1(4): 522-533.
11. Karthika K, Paulsamy S, Jamuna S. Evaluation of *in vitro* antioxidant potential of methanolic leaf and stem extracts of *Solena amplexicaulis* (Lam.) Gandhi. Journal of chemical and pharmaceutical research 2012; 4(6): 3254-3258.
12. Pullaiah T, Murthy KSR, Goud PSP, Kumar TDC, Vijayakumar R. Medicinal plants used by the tribals of Nallamalais, Eastern Ghats of India. Journal of tropical medicinal plants 2003; 4(2): 237-244.
13. Bauer RW, Kirby MDK, Sherris JC, Turek M. Antibiotic susceptibility testing by standard single disc diffusion method. American journal of clinical pathology 1966; 45: 493-496.
14. Kim J, Marshall MR, Wei C. Antibacterial activity of some essential oil components against five food borne pathogens. Journal of Agricultural and Food Chemistry 1995; 43: 2839-2845.
15. Muhamed Mubarack H, Doss A, Dhanabalan R, Venkataswamy R. Activity of some selected medicinal plant extracts against bovine mastitis pathogens. Journal of Animal and Veterinary Advances 2011; 10(6): 738-741.
16. Rani O, Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi drug resistant *Salmonella typhi.* Phytotherapy Research. 2004; 18: 670-673.
17. Finberg RW, Moellering RC, Tally FP, Craig WA, Pankey GA, Dellinger EP *et al.* The importance of bactericidal drugs: future directions in infectious disease. Clinical Infectious Diseases 2004; 39(9): 1314-doi: 10.1086/425009. PMID 15494908.
18. Cunha BA. Antibiotic Essentials. Jones and Bartlett Learning, ISBN 978-0-7637-7219-2. 180.
19. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. Complementary and Alernative Medicine 2006; 6: 35.
20. Girish HV, Satish S. Antibacterial activity of important medicinal plants of human pathogenic bacteria - a comparative analysis. World Applied Sciences Journal 2008; 5(3): 267-271.
21. Tekeli Y, Zengin G, Aktumsek A, Sezgin M, Torlak E. Antibacterial activities of extracts from twelve *Centaurea* species from Turkey. Archives of Biological Sciences 2011; 63(3): 685-690.
22. Arawwawala LDAM, Thabraw MI, Arambewela LSR, Fernando N, Guruge LD. Antibacterial activity of *Trichosanthes cucumerina* Linn. extracts. International journal of pharmaceutical and biological archives 2011; 2(2): 808-812.
23. Gurudeeban S, Rajamanickam E, Ramanathan T, Satyavani K. Antimicrobial activity of *Citrullus colocynthis* in gulf of mannar. International journal of current research 2010; 2: 078-081.
24. Farrukh U, Shareef H, Mahmud S, Ali SA, Rizwani GH. Antibacterial activity of *Coccinia grandis* L. Pakistan Journal of Botany 2008; 40(3): 1259-1262.