

Original Article

ANTIMICROBIAL ACTIVITY AND TLC PROFILING OF *CLERODENDRUM PHLOMIDIS* LINN. F LEAF EXTRACT AGAINST MULTI-DRUG RESISTANT CLINICAL PATHOGENS

A. HANNAH HEPSIBAH, M. MALA, G. JEYA JOTHI*

Department of Plant Biology and Biotechnology, Loyola College, Chennai 600034, Tamil Nadu, India
Email: gjothiloyola@gmail.com

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ABSTRACT

Objective: The aim of the present study is to evaluate the antibacterial and antifungal activities of different solvent extracts of leaves of *Clerodendrum phlomidis* against multi-drug resistant clinical pathogens and to perform Thin Layer Chromatography (TLC) profiling of the extracts.

Methods: The leaf powder was sequentially extracted with petroleum ether, ethyl acetate, acetone, and methanol. The extracts were tested for their antimicrobial activity against 20 bacterial strains and 15 fungal strains which were multiple drugs resistant by disc diffusion method. TLC profiling of all four extracts was performed.

Results: The petroleum ether and ethyl acetate extracts showed antimicrobial activity against four bacteria and seven fungal strains. The highest zone of inhibition (18 ± 0.50 mm) for bacteria was recorded against *Staphylococcus epidermis* by ethyl acetate extract and for fungi, the petroleum ether extract showed 13 ± 0.00 mm against *Trichophyton rubrum*. TLC profiles showed that ethyl acetate extract possessed more compounds.

Conclusion: From the results of the present study it is concluded that the petroleum ether and ethyl acetate extracts of *C. phlomidis* leaves are good sources of antimicrobial compounds which can inhibit multiple drug resistant pathogens.

Keywords: Antimicrobial activity, Disc diffusion, Multiple drug resistant pathogens, TLC profiling, *Clerodendrum phlomidis*

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INTRODUCTION

Medicinal plants are used throughout the world for various ailments in Ayurveda, Siddha and other forms of traditional medicines. They are cost-effective and have minimal side effects which make them an alternative form of health care [1]. In recent past, there has been a rise in microbial resistance to the existing antibiotics which has turned the attention of researchers towards the natural antimicrobial compounds present in medicinal plants [2].

Plant phytochemicals target the biochemical pathway, hence are safer than synthetic drugs. Medicinal plants are of great value in developing countries where infectious disease are endemic and modern health facilities and services are inadequate [3].

Clerodendrum phlomidis Linn. f. (syn. *Clerodendrum multiflorum*) belongs to the family Verbenaceae. It is commonly known as wind killer in English and Talludhalai in Tamil. *C. phlomidis* is a common shrub found in arid plains, low hills and tropical deserts [4]. Its leaves and roots are used in folklore, Ayurveda, Siddha and Unani medicines. The roots and leaves are used for the treatment of rheumatism, asthma, inflammation, diabetes, nervous disorders, digestive disorders, urinary disorders and also as a pesticide in agriculture [5]. This plant has been studied for its antimicrobial, antioxidant, antimalarial, antidiabetic and anti-glycemic activities [5, 10]. As the plant possesses antimicrobial activity it was considered as a good candidate to prove its potency against multi-drug resistant clinical isolates.

The present study was aimed to evaluate the antibacterial and antifungal actives of the leaf extracts of *C. phlomidis* against multi-drug resistant clinical pathogens and to perform TLC profiling of the extracts.

MATERIALS AND METHODS

Collection of plant material

The plant *C. phlomidis* leaves were collected from Karaiyar (Papanasam), Tirunelveli District, Tamil Nadu, during the month of

June 2016. The plant was identified by the taxonomist Dr. G. Jeya Jothi and a Voucher specimen (439) was deposited in the Loyola College Herbarium.

Extraction

The healthy and disease-free leaves were collected, washed under running tap water and dried under shade at room temperature. The dried leaves were powdered in a blender and stored in an airtight container for further use. The ground leaf powder was serially extracted with petroleum ether, ethyl acetate, acetone, and methanol in 1:4 ratios for 72 h each. The obtained crude extracts were concentrated by Rotary evaporator at reduced pressure. The solvent-free extracts were used for further experimental studies.

Chemicals

All solvents used for extraction and the TLC plates were purchased from Merk, Germany. The media for antimicrobial activity were procured from Himedia, Mumbai, India.

Microorganisms

For the present study, 20 bacterial and 15 fungal cultures were chosen. All the organisms were clinical isolates obtained from CMC, Vellore.

The bacteria used in the study were *Micrococcus luteus*, *Staphylococcus epidermis*, *Yersinia enterocolitica*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Mucoid pneumoniae*, *Salmonella paratyphi*, *Enterococcus faecalis*, *Serratia marcescens*, *Methicillin-resistant Staphylococcus aureus* and *Acinetobacter*.

The fungi used in this study were *Scedosporium sp.*, *Phialophora verrucosa*, *Aspergillus terreus*, *Trichophyton rubrum*, *Rhizopus sp.*, *Fusarium sp.*, *Scytalidium dimidiatum*, *Paecilomyces sp.*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Candida albicans*, *Candida krusei* and *Candida tropicalis*.

Antimicrobial activity

The Disc diffusion method was used to screen the antimicrobial activity [6, 7]. The Agar plates were inoculated with the test organism; 25 μ l of the extract was dissolved in Di Methyl Sulfoxide (DMSO) and loaded on sterile discs. The discs were placed on the Petri plates inoculated with the test organism and plates were kept for incubation at 37 °C for 24 h for bacteria and 48 h for fungi. The inhibition zones formed were measured in millimetres. The experiment was done in triplicates, and the mean value of the zone of inhibition was calculated. Ceftazidime (30 μ g), Vancomycin (10 μ g), Methicillin (10 μ g) and Ciprofloxacin (5 μ g) were used as positive controls for bacteria and Clotrimazole (10 μ g) and Fluconazole (10 μ g) were used as positive control for fungi. DMSO was taken as negative control.

Thin layer chromatography

Moisture free TLC plates (5 \times 10 cm) were taken. Each solvent extract which was diluted with appropriate solvent was loaded 1 cm away from the base of the TLC plate using a capillary tube and the samples were allowed to dry. Different solvent systems were used as mobile phase, they were: 1) Hexane: Ethyl acetate, 2) Chloroform: Methanol, 3) Ethyl acetate: Methanol and 4) Toluene: Ethyl acetate in different ratios ranging from 9:1 to 5:5 each. The TLC plates were allowed to develop in a closed chamber containing the mobile phase after which the plates were thoroughly dried. The plates were observed under white light and UV lamp (254 nm, 366 nm wavelength). The

plates were finally stained with Vanillin: H₂SO₄ (1 gm vanillin and 10 ml of H₂SO₄ in 190 ml of ethanol) and heated at 100 °C to develop colored bands. Different color bands were noted and R_f values were calculated.

Statistical analysis

All analysis was repeated thrice and the results were presented as mean \pm SD.

RESULTS

All four plant extract were tested against 20 bacterial strains and 15 fungal strains by disc diffusion method (table 1, table 2). Only petroleum ether and ethyl acetate extracts showed activity whereas acetone and methanol extracts did not show any activity against any of the microbial strains.

Antibacterial activity

Among the 20 Antibiotic Resistant bacterial strains tested, only 4 were susceptible to the petroleum ether and ethyl acetate extracts. The highest zone of inhibition (18 \pm 0.50 mm) was seen against *S. epidermis* for ethyl acetate extract, followed by a zone of 11 \pm 0.50 mm against *MRSA* bacteria by ethyl acetate extract and against *St. pyogenes* by petroleum ether extract. The petroleum ether extract showed an inhibition zone of 10 \pm 0.25 mm and 10 \pm 0.00 mm against *S. epidermis* and *Se. marcescens* respectively.

Table 1: Antibacterial activity of solvent extracts of *Clerodendrum phlomidis* leaves

S. No.	Bacterial strain	Zone of inhibition(mm) ^a				Activity of the standard (mm) ^a			
		PE	EA	A	M	CAZ	MET	VA	CIP
1	<i>Micrococcus luteus</i>	-	-	-	-	16 \pm 0.00	-	-	14 \pm 0.15
2	<i>Staphylococcus epidermis</i>	10 \pm 0.25	18 \pm 0.50	-	-	-	-	-	-
3	<i>Yersinia enterocolitica</i>	-	-	-	-	14 \pm 0.10	-	-	13 \pm 0.25
4	<i>Enterobactor aerugenes</i>	-	-	-	-	-	-	-	17 \pm 0.15
5	<i>Salmonella typhimurium</i>	-	-	-	-	-	-	-	-
6	<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	08 \pm 0.10
7	<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	17 \pm 0.50
8	<i>Streptococci pneumoniae</i>	-	-	-	-	-	-	-	-
9	<i>Streptococcus pyogenes</i>	11 \pm 0.00	-	-	-	8 \pm 0.50	-	-	10 \pm 0.00
10	<i>Streptococcus agalactiae</i>	-	-	-	-	12 \pm 0.00	-	-	08 \pm 0.10
11	<i>Escherichia coli</i>	-	-	-	-	-	-	-	-
12	<i>Pseudomonas aeriginosa</i>	-	-	-	-	-	-	-	16 \pm 0.50
13	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-
14	<i>Shigella dysenteriae</i>	-	-	-	-	-	-	-	9 \pm 1.00
15	<i>Mucoid pneumonia</i>	-	-	-	-	13 \pm 0.00	8 \pm 0.50	-	-
16	<i>Salmonella paratyphi</i>	-	-	-	-	-	-	-	11 \pm 0.00
17	<i>Enterococcus faecalis</i>	-	-	-	-	-	7 \pm 0.00	-	12 \pm 0.20
18	<i>Serratia marcescens</i>	10 \pm 0.00	-	-	-	12 \pm 0.50	10 \pm 0.50	-	13 \pm 0.25
19	<i>Methicillin-resistant Staphylococcus aureus</i>	-	11 \pm 0.50	-	-	-	-	-	-
20	<i>Acinetobactor</i>	-	-	-	-	10 \pm 0.10	-	-	10 \pm 1.00

^amean \pm SD, n=3; mm-Milli Meter, PE-Petroleum Ether, EA-Ethyl Acetate, A-Acetone and M-Methanol; CAZ-Ceftazidime (30 μ g),MET-Methicillin (10 μ g),VA-Vancomycin (10 μ g), CIP-Ciprofloxacin(5 μ g).

Antifungal activity

Among the 15 fungal strains which were Fluconazole and Clotrimazole resistant, seven were susceptible to the petroleum ether and ethyl acetate extracts. Petroleum ether extract showed the highest zone of 13 \pm 0.00 mm against *Trichophyton rubrum*, followed by ethyl acetate which showed a zone of inhibition of 11 \pm 0.00 mm and 10 \pm 0.25 mm against *A. niger* and *Scedosporium sp.* respectively.

Thin layer chromatography profiles

All the TLC plates were stained with Vanillin Sulphuric acid and heated at 100 °C to develop coloured bands. The plates showing proper separation were observed and their R_f value was calculated (table 3). Ethyl acetate extract showed the maximum number of separation followed by acetone, petroleum ether, and methanol. The formation of coloured bands was attributed to different phytochemical groups [8].

Formation of brown colour indicates the presence of essential oils in petroleum ether and ethyl acetate extracts. The blue and violet bands show the presence of saponins in all four extracts. The ethyl acetate and acetone extracts showed the presence of bitter principals (yellow bands), phenolic compounds (green) and terpenes (pink).

DISCUSSION

Throughout the world, more and more clinical microbes are becoming resistant to all major antibiotics available and according to the WHO no new antimicrobial drugs have been developed over the last 30 y [9]. Hence, this has made us turn our attention towards traditional medicines and medicinal plants.

C. phlomidis is a widely studied medicinal plant. It possesses antimicrobial, antioxidant, antimalarial, antidiabetic and anti-glycemic activities [5, 10]. Since it has been already proven to

possess antimicrobial activity this plant was chosen to evaluate its potency against Multi-Drug resistant clinical isolates. The solvents for extraction were selected based on their polarity, from non-polar

to highly polar solvents in order to perform serial extraction, which will lead to the extraction of a wide range of compounds with varied polarity [11].

Table 2: Shows antifungal activity of various solvent extracts of *Clerodendrum phlomidis*

S. No.	Fungal strains	Zone of inhibition(mm) ^a				Activity of standard(mm) ^a	
		PE	EA	A	M	FLC	CC
1	<i>Scedosporium sp.</i>	8±0.10	10±0.25	-	-	-	-
2	<i>Phialophora verrucosa</i>	-	-	-	-	-	-
3	<i>Aspergillus terreus</i>	-	8±0.00	-	-	-	-
4	<i>Trichophyton rubrum</i>	13±0.00	-	-	-	-	-
5	<i>Rhizopus sp.</i>	-	-	-	-	-	-
6	<i>Fusarium sp.</i>	-	-	-	-	-	-
7	<i>Scytalidium dimidiatum</i>	7±0.50	8±0.10	-	-	-	-
8	<i>Paecilomyces sp.</i>	-	7±0.50	-	-	-	-
9	<i>Aspergillus fumigatus</i>	-	-	-	-	-	-
10	<i>Aspergillus niger</i>	9±0.00	11±0.00	-	-	-	-
11	<i>Aspergillus flavus</i>	7±1.00	8±0.00	-	-	-	-
12	<i>Fusarium oxysporum</i>	-	-	-	-	-	-
13	<i>Candida albicans</i>	-	-	-	-	-	-
14	<i>Candida krusei</i>	-	-	-	-	-	-
15	<i>Candida tropicalis</i>	-	-	-	-	-	-

^amean±SD, n=3; mm-Milli Meter, PE-Petroleum Ether, EA-Ethyl Acetate, A-Acetone and M-Methanol; FLC-Fluconazole (10 µg), CC-Clotrimazole (10 µg).

Table 3: TLC analysis for solvent extracts of *Clerodendrum phlomidis* leaves

Extract	Solvent system	No. of bands	Colour	Rf value
Petroleum Ether	H: EA(9:1)	12	Blue Brown	0.200.97
	T: EA(7:3)	12	VioletBrown	0.540.87
Ethyl Acetate	H: EA(7:3)	123456	YellowVioletPinkYellowBlueBrown	0.320.540.560.720.780.97
	T: EA(5:5)	12345	BlueGreenYellowVioletGreen	0.010.210.720.780.87
	EA: M(9:1)	12345	GreenVioletLight	0.560.640.700.740.81
Acetone	C: M(5:5)	123456	GreenGreenViolet	
	H: EA(7:3)	12	BluePinkBlueGreenYellowBlue	0.080.300.350.410.880.90
	T: EA(5:5)	1234	YellowPink	0.340.54
	EA: M(9:1)	123	BlueGreenYellowViolet	0.180.230.720.78
	C: M(5:5)	12345	VioletLight GreenGreen	0.640.700.74
Methanol	C: M(5:5)	12345	BlueBlueGreenYellowBlue	0.080.350.410.880.90
	C: M(5:5)	12	BlueBlue	0.080.35

Ethyl acetate extract showed good activity against both bacterial and fungal strains and also showed the presence of the highest number of compounds in the TLC profile. Petroleum ether extract, on the other hand, showed good activity but fewer amounts of bands. Acetone extract did not show any activity but showed the second highest separation of extracts.

This shows that that the antimicrobial compounds of *C. phlomidis* should be either nonpolar or mid-polar because only petroleum ether and ethyl acetate showed antimicrobial activity.

Earlier studies have shown that ethyl acetate extract has good antifungal activity and petroleum ether, ethanol, and chloroform extracts showed good antibacterial activity [4].

Previous studies on GC-MS of petroleum ether extract showed the presence of Isopropyl linoleate, Hexadecanoic acid, 2-Hydroxyl-1-[Hydroxymethyl] Ethyl Ester, 9-Octadecenoic acid[Z]-, 2-Hydroxy-1-[Hydroxymethyl]Ethyl Ester and that of ethyl acetate extract showed the presence of 1,11-Tridecadiene [12]. These compounds may attribute to the biological activity of the extracts.

In the present study, essential oils were extracted only in the active extracts namely petroleum ether and ethyl acetate extracts. Earlier studies have shown the presence of various classes of steroids and terpenes and flavonoids from *C. phlomidis* [13]. Pectolinarigenin and chalcone glucoside isolated from the leaf of the *C. phlomidis* plant showed antifungal activity [5].

CONCLUSION

From the present study, we conclude that the petroleum ether and ethyl acetate extracts of *C. phlomidis* leaves are a very good source of antimicrobial compounds which can inhibit multiple drug resistant organisms. The next step would be to isolate and identify the target compound using the appropriate solvent and the appropriate method.

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AUTHOR CONTRIBUTION

A. Hannah Hepsibah and M. Mala contributed towards the collection of plant material, experimental design, performing the experiment, data compilation and statistical analysis. Dr. G. Jeya Jothi is the Guide and Principal Investigator of the ICMR project and guided and monitored the experimental design, data compilation and statistical analysis and corrected the manuscript.

CONFLICT OF INTERESTS

Declared none

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