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Research Article

Dicentra scandens (D.Don) Walp. - A Potential Source of Antimicrobial Agent

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ABSTRACT

Dicentra scandens (D.Don) Walp. locally called as '*Jogi Lahara*' belongs to the Family *Fumariaceae* and is used in traditional medicine in Sikkim, a North Eastern state of India. The present study investigated the antimicrobial properties of aqueous and methanol extracts of *Dicentra scandens* (D.Don) Walp. against some test Gram negative and Gram positive bacteria. Using agar well diffusion method, aqueous and methanol extracts of roots of *Dicentra scandens* were tested against *Escherichia coli* (MTCC 1089), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 1034), *Proteus vulgaris* (MTCC 742), *Salmonella typhi* (MTCC 733), *Shigella flexneri* (MTCC 1457), *Vibrio cholerae* O139(MTCC 3906), *Bacillus cereus* (MTCC 6840) and *Staphylococcus aureus* (MTCC 7443). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were also evaluated. Promising antimicrobial activity was exhibited by methanol extract of *Dicentra scandens*. The methanol extract was further characterized by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The GC-MS analysis revealed 29 compounds and the major compounds detected were Protopine (53.78%) and Corydine (18.20%). Thus, the alkaloids are predominant phytoconstituents of the extract and could be attributed to its antimicrobial activity. The results of the present study indicate that, *Dicentra scandens* can be a source of potential antimicrobial agent and can be explored further for its therapeutic use.

Keywords: Alkaloid, Antimicrobial, Corydine, *Dicentra scandens*, GC-MS analysis, Protopine

INTRODUCTION

Dicentra scandens (D. Don) Walp. is a climbing perennial herb with tuberous rootstock[1], belonging to the family *Fumariaceae.* It is distributed in temperate Northern Asia and North America[1]. Various tribal communities of Nepal and North East India traditionally use *Dicentra scandens* (D. Don) Walp. as an important ethnomedicinal plant[2]. The herbalist from Nagaland use this plant for the treatment of malaria, typhoid, viral fever, diabetes, pneumonia, high blood pressure, diarrhoea, dysentery, flatulence, cut or injury[3]. Crushed tuberous root with water is taken against fever, stomach ache and high blood pressure in Manipur[4]. Antibacterial and antifungal activity of n- hexane, ethyl acetate and ethanolic extracts of root of *Dicentra scandens* against potential wound pathogens has been investigated[5]. The root juice of *Dicentra scandens* is useful in gastritis. Leaf paste or juice is applied on cuts and wounds[6]. Dried root powder of *Dicentra scandens* is administered orally during gastritis by the *Limboo* tribe residing in South-West Khangchendzonga biosphere reserve in West Sikkim, India[7]. Ecologically, Yonzone, et al. (2012) categorised *Dicentra scandens* as a rare medicinal herb found in Darjeeling Himalaya of West Bengal, India[8]. The present study aims to document and investigate the antimicrobial property of *Dicentra scandens* (D. Don) Walp. collected from Damthang, South district of Sikkim, India.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used including solvents were obtained from Sigma-Aldrich, USA, Merck, Germany and HiMedia, India and were of analytical grade.

Collection and identification of plant sample

Tuberous roots of *Dicentra scandens* were collected from Damthang (27°13'60' N) South District of Sikkim (India). A voucher specimen (Accession number 09734) was deposited at the herbarium of the Taxonomy Division, Department of Botany, University of North Bengal, India.

Preparation of crude methanol and aqueous extracts

The root of *Dicentra scandens* were cut in small pieces and dried in shade. The dried plant material was ground into powder using Waring blender (Cole Parmer, RZ-04245-21). The powdered material 20grams each was extracted with 200ml of respective solvents (aqueous and methanol) for 24 hours using orbital shaker. The solvent was then evaporated under reduced pressure in a Rotary evaporator (Buchi, Switzerland, R-3). The concentrated extract was stored at 4°C till further analysis[9]. Prior to antibacterial assay, extracts were dissolved in 0.25% dimethyl sulphoxide (DMSO).

Phytochemical analysis

Aqueous and methanol extracts of *Dicentra scandens* were subjected to qualitative phytochemical analyses to examine the phytoconstituents namely phenol, flavonoid, tannin, saponin, steroid, anthocyanin, alkaloid, glycoside, carbohydrate, protein and fat[10,11,12,13].

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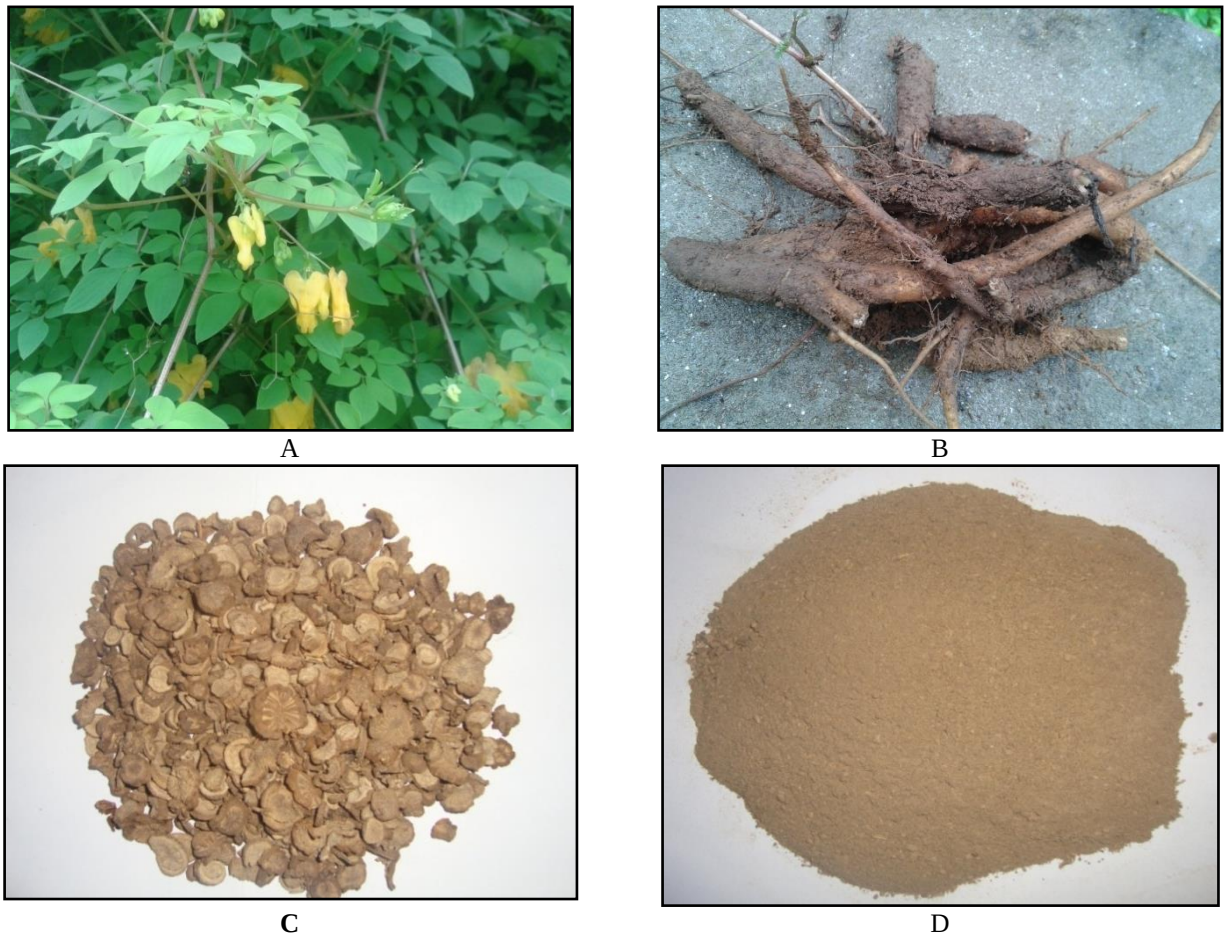


Figure 1: A. *Dicentra scandens* (D.Don) Walp. B. Tuberous root C. Dried sample from tuberous root D. Powdered sample.

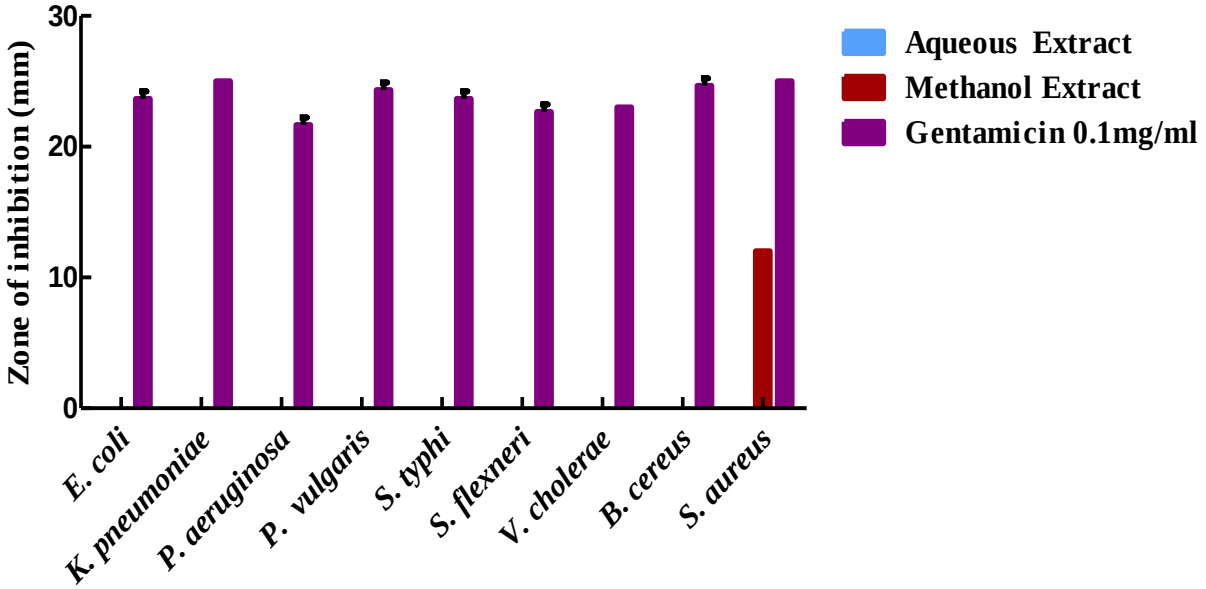


Figure 2: Zone of inhibition against different test microorganisms by aqueous and methanol extracts of *Dicentra scandens* at the concentration of 25mg/ml.
The data represents mean ± SD. All the experiments were performed in triplicate.

Table 1: Minimum Inhibitory Concentration, Minimum Bactericidal Concentration in mg/ml and MIC index of the extracts against test bacterial strains.

		Aqueous extract	Methanol extract
Escherichia coli	MIC	-	25
	MBC	-	50
	MIC Index	-	2
Klebsiella pneumonia	MIC	-	100
	MBC	-	100
	MIC Index	-	1
Pseudomonas aeruginosa	MIC	-	100
	MBC	-	100
	MIC Index	-	1
Proteus vulgaris	MIC	50	50
	MBC	200	100
	MIC Index	4	2
Salmonella typhi	MIC	-	25
	MBC	-	100
	MIC Index	-	4
Shigella flexneri	MIC	-	12.5
	MBC	-	50
	MIC Index	-	4
Vibrio cholerae O139	MIC	-	6.25
	MBC	-	6.25
	MIC Index	-	1
Bacillus cereus	MIC	-	100
	MBC	-	200
	MIC Index	-	2
Staphylococcus aureus	MIC	100	25
	MBC	200	100
	MIC Index	2	4

'-'absence of zone of inhibition in agar well diffusion method at higher concentration (400mg/ml).

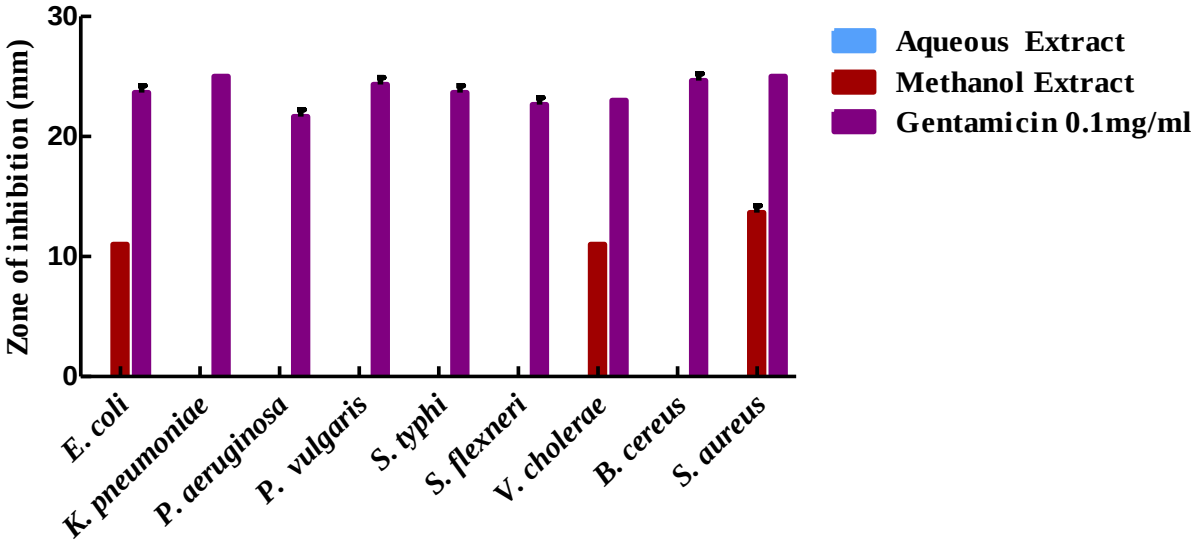


Figure 3: Zone of inhibition against different test microorganisms by aqueous and methanol extracts of *Dicentra scandens* at the concentration of 50mg/ml.
The data represents mean ± SD. All the experiments were performed in triplicate.

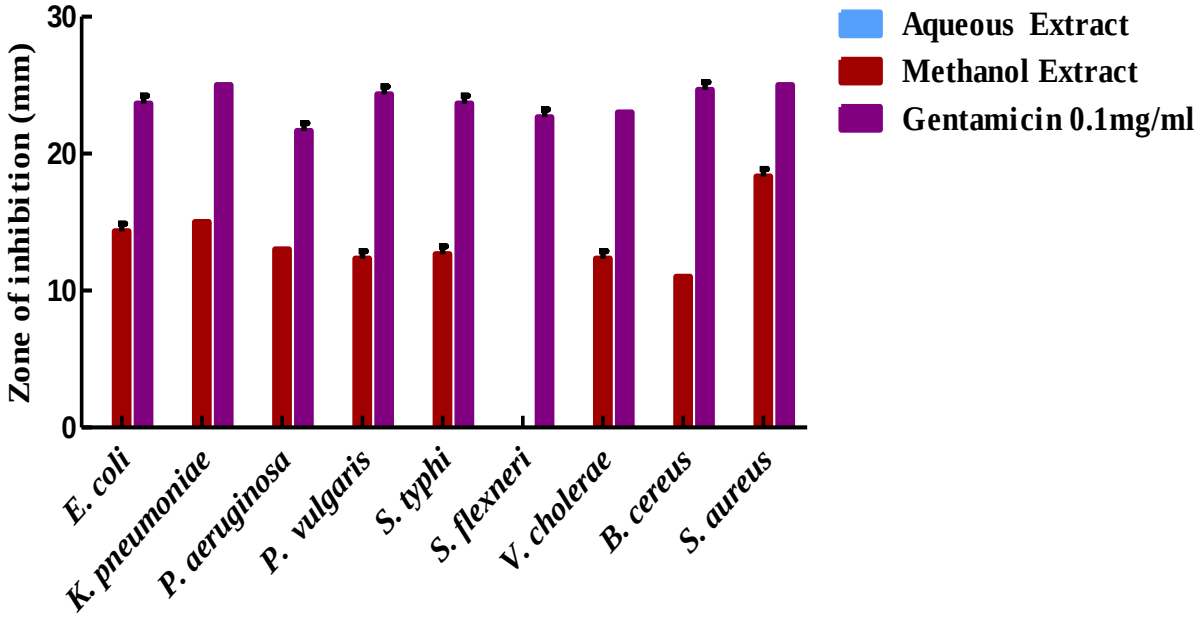


Figure 4: Zone of inhibition against different test microorganisms by aqueous and methanol extracts of *Dicentra scandens* at the concentration of 100mg/ml.
The data represents mean ± SD. All the experiments were performed in triplicate.

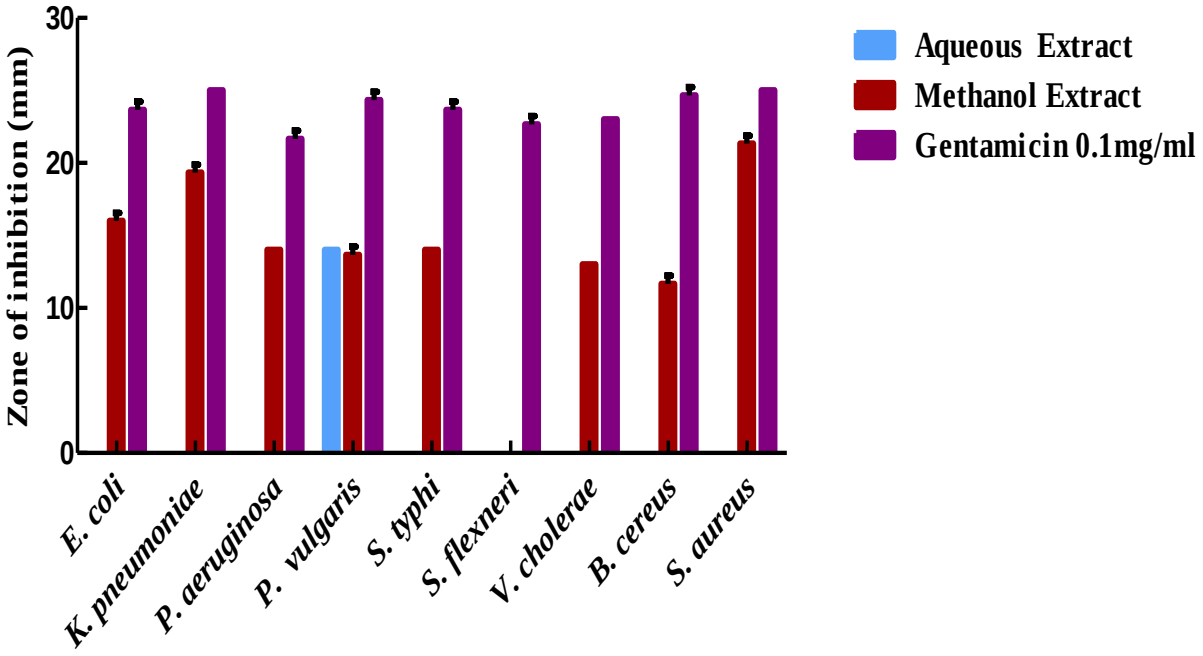


Figure 5: Zone of inhibition against different test microorganisms by aqueous and methanol extracts of *Dicentra scandens* at the concentration of 200mg/ml.
The data represents mean ± SD. All the experiments were performed in triplicate.

Determination of total phenolic content

Total phenolic content was determined according to Folin-Ciocalteu method[14] using gallic acid as the standard. Total phenolic content was represented as mg of gallic acid equivalent (GAE) per gram of extract. The entire test was done in triplicate and their mean value was represented.

Determination of total flavonoid content

The total flavonoid content was estimated by aluminium chloride colorimetric method[15] using rutin as a standard reference. Total flavonoid content was expressed as mg of rutin equivalent (RE) per gram of extract. The entire test

Table 2: Total phenolic, flavonoid and tannin content of aqueous and methanol extracts of *Dicentra scandens*.

Extracts of *Dicentra scandens*	Total phenolic content (mg GAE/g) ± SD	Total flavonoid content (mg RE/g) ± SD	Total tannin content (mg TAE/g) ± SD
Methanol extract	12.405 ± 0.037	2.366 ± 0.057	23.920 ± 0.036
Aqueous extract	5.052 ± 0.200	3.166 ± 0.246	19.240 ± 0.017

Each value is expressed as mean ± SD. GAE; gallic acid equivalent, RE; rutin equivalent, TAE; tannic acid equivalent. All the experiments were performed in triplicate.

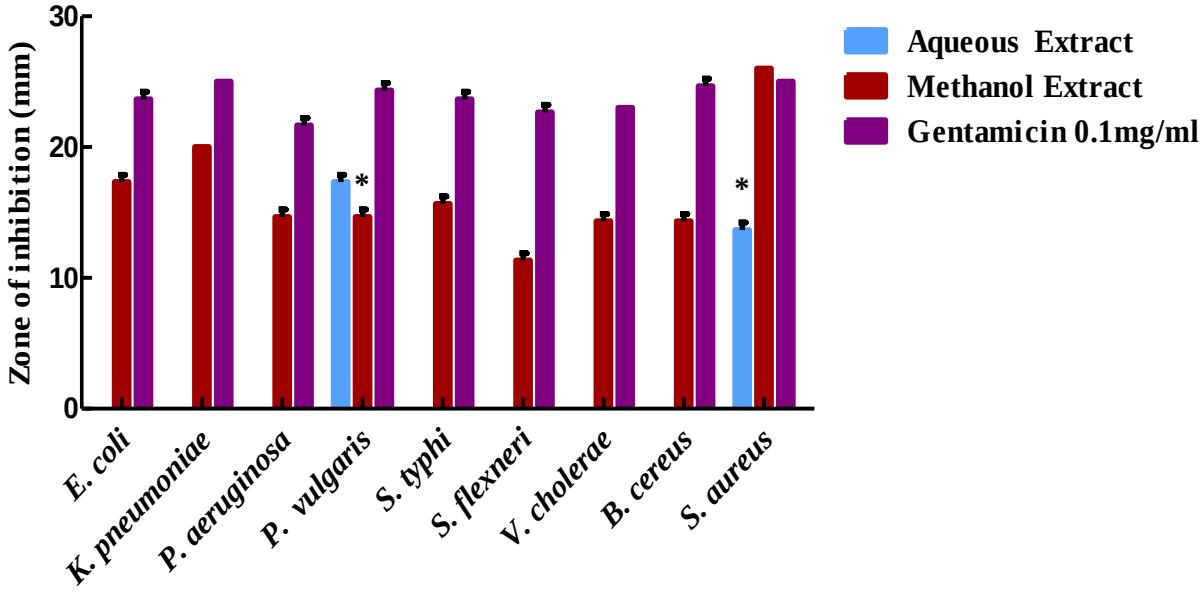


Figure 6: Zone of inhibition against different test microorganisms by aqueous and methanol extracts of *Dicentra scandens* at the concentration of 400mg/ml.
*Methanol vs. Aqueous (P<0.05). The data represents mean ± SD. All the experiments were performed in triplicate.

was done in triplicate and their mean value was represented.

Determination of total tannin content

Total tannin content was determined by Folin Denis spectrophotometric method using tannic acid as reference standard[16]. Total tannin content was expressed as mg of tannic acid equivalent (TAE) per gram of extract. The entire test was done in triplicate and their mean value was represented.

Determination of antimicrobial activity

The antimicrobial assay was performed by following standard agar well diffusion method[17]. The test bacteria used in this study including *Escherichia coli* (MTCC 1089), *Klebsiella pneumoniae* (MTCC-3384), *Pseudomonas aeruginosa* (MTCC-1034), *Proteus vulgaris* (MTCC-742), *Salmonella typhi* (MTCC 733), *Shigella flexneri* (MTCC 1457), *Vibrio cholerae* O139 (MTCC 3906), *Bacillus cereus* (MTCC- 6840) and *Staphylococcus aureus* (MTCC-7443) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The test plant extracts were dissolved in 0.25% dimethyl sulphoxide (DMSO) to final stock concentrations of 400mg/ml and sterilized by filtration through 0.45 µm cellulose acetate membrane filters (Sartorius). Various concentrations (25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml, 400mg/ml) of aqueous and methanol extracts were prepared using stock concentration. DMSO (0.25%) was used as the negative control. Gentamicin (0.1mg/ml)[18] was used as the positive control. Antimicrobial activity was determined by measuring the diameter of inhibition zone (DIZ)[19] inclusive of well diameter of 8mm. All the tests were performed in triplicate. The extracts with antibacterial activity were serially diluted for concentrations ranging from 400–0.048 mg/ml for determination of their minimum inhibitory concentration (MIC) according to the method of Wiegand et al. (2008)[20]. Minimum bactericidal concentration was determined according to the method described by Heredia et al. (2005)[21].

Gas Chromatography-Mass Spectrometry analysis

The extract which showed maximum antimicrobial activity, specifically methanol extract, was subjected to GC-MS analysis on a GCMS- QP210 Plus system (Shimadzu), at the Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India. In case of GC-MS analysis the interpretation for the mass spectrum analysis was done using the database of the National Institute of Standard Technology (NIST11)

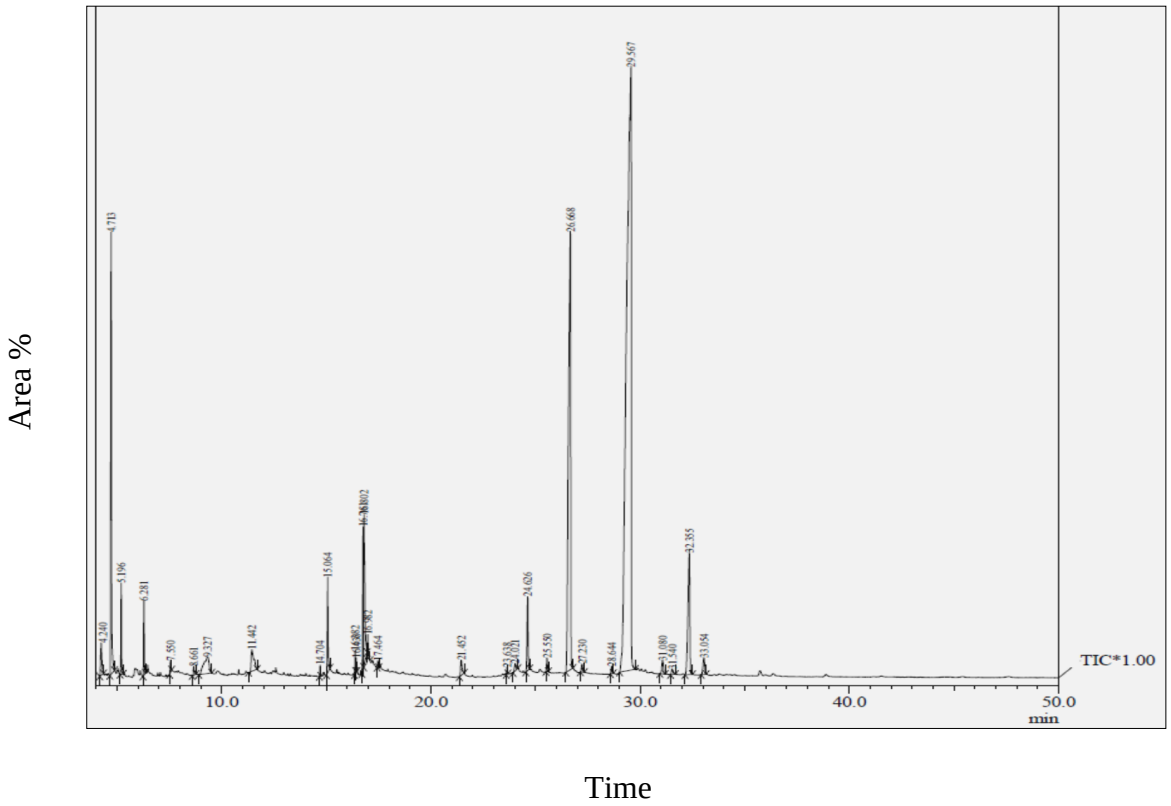


Figure 7: Gas Chromatography-Mass Spectrometry chromatogram of methanol extract of *Dicentra scandens*.

library and WILEY8 library as provided by the Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism V5.01 (San Diego, USA). The data were analysed using Two-Way ANOVA. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The present study investigated the antimicrobial activity of *Dicentra scandens* (D. Don) Walp. collected from Damthang, South district of Sikkim, India.

Antimicrobial activity of aqueous and methanol extracts of roots of *Dicentra scandens* was evaluated against nine test microorganisms namely *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Shigella flexneri*, *Vibrio cholerae* O139, *Bacillus cereus* and *Staphylococcus aureus*. The methanol extract exhibited significantly ($p<0.05$) higher antimicrobial activity as compared to the aqueous extract. The methanol extract was found to be effective against *Staphylococcus aureus* at the concentration of 25mg/ml which was the lowest concentration used for antimicrobial assay in our study (Figure 2). At the concentration of 50mg/ml, the extract exhibited antimicrobial activity against *Escherichia coli*, *Vibrio cholerae* and *Staphylococcus aureus* (Figure 3). At the concentrations of 100mg/ml and 200mg/ml, the methanol extract inhibited the growth of most of the test microorganisms except *Shigella flexneri* (Figure 4 and Figure 5). However, at the concentration of 400mg/ml the methanol extract exhibited antimicrobial activity against all the nine test microorganisms with the largest zone of inhibition (26 mm) observed in *Staphylococcus aureus* (Figure 6). On the other hand, at the concentration of 400mg/ml the aqueous extract inhibited the growth of two test microorganisms namely *Proteus vulgaris* and *Staphylococcus aureus* (Figure 6). It is interesting to note that the zone of inhibition (17.33 ± 0.57 mm) formed by aqueous extracts against *Proteus vulgaris* was significantly ($p<0.05$) higher than that of the methanol extract (Figure 6). At a concentration of 200mg/ml the aqueous extract inhibited the growth of only *Proteus vulgaris* (Figure 5). For aqueous extract the MIC values were comparatively higher with 50mg/ml for *Proteus vulgaris* and 100mg/ml for *Staphylococcus aureus*. The MBC value was 200mg/ml for both the test microorganisms. In case of methanol extract the MIC and MBC values ranged from 6.25mg/ml to 100mg/ml and 6.25mg/ml to 200mg/ml respectively (Table 1). Based on the MIC index value which was ≤ 4, both the extracts were found to be bactericidal in nature. The value of MIC index determines whether the plant extract is bactericidal or bacteriostatic in nature. When the MIC index is ≤ 4, the extract is bactericidal and when the MIC index is >4, the extract is bacteriostatic[22].

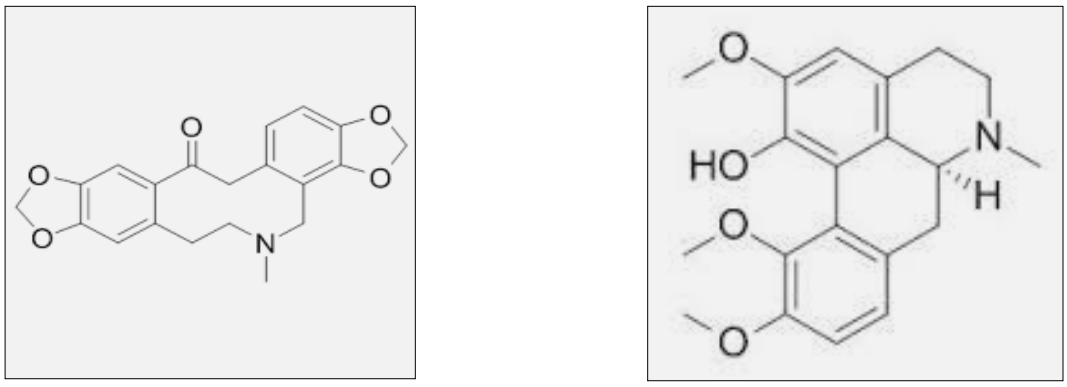
The qualitative phytochemical analysis of the aqueous and methanol extracts revealed the presence of flavonoid, anthocyanin, phenol, tannin, alkaloid, steroid and

Table 3: Components (more than 1 % peak area) detected by GC-MS analysis in methanol extract of *Dicentra scandens.*

Sl. No.	Retention Time	% Area	Compound Name	Formula	Molecular weight	Compound Nature	Biological activity
1	5.196	1.13	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One	$C_6H_8O_4$	144	Flavonoid	Antimicrobial, anti-inflammatory[29]
2	11.442	1.09	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192	Quinic acid	Antimicrobial, anti-inflammatory, antioxidant [30]
3	15.064	1.34	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	Lauric acid	Antioxidant[31]
4	16.761	1.99	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	Trans-Linoleic acid	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective[29, 31]
5	16.802	3.27	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	Omega-7 fatty acid	Cosmetics[32]
6	24.626	1.51	Hordenine	$C_{10}H_{15}NO$	165	Alkaloid	Anti-cholinesterase Activity[33]
7	26.668	18.20	Corydine	$C_{20}H_{23}NO_4$	341	Alkaloid	DNA damaging activity [27]
8	29.567	53.78	Protopine	$C_{20}H_{19}NO_5$	353	Alkaloid	Antibacterial, antiviral, antifungal, antiparasitic, antithrombotic, anti-inflammatory, anti-spasmodic, neuroprotective[26]

glycoside. Saponin was detected only in the aqueous extract. In the quantitative analysis, the methanol extract was found to contain comparatively higher amount of total tannin content (23.920 ± 0.036 mg TAE/g) (Table 2). Presence of high amount of tannin may have contributed to the antimicrobial activity of the methanol extract of *Dicentra scandens*. Tannin exerts its antimicrobial effects by inhibition of extracellular microbial enzymes, by interfering with the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation[23]. Similarly, the highest phenolic content (12.405 ± 0.037 mg GAE/g) was observed in the methanol extract (Table 2). Hence the presence of high amount of phenolic compounds in combination with other phytochemicals in the methanol extract may have contributed for its higher antimicrobial activity as compared to the aqueous extract. Further, methanol has been reported to exhibit better extraction efficiency than other solvents[24].

Figure 7 depicted the GC-MS chromatogram of the methanol extract of *Dicentra scandens.* The GC-MS analysis revealed twenty nine compounds. The major compounds detected were Protopine (53.78%) and Corydine (18.20%) (Table 3). Both the compounds detected were alkaloids and possess various biological activities (Table 3). Alkaloids are the nitrogenous compound which interferes with cell division thereby exerting antimicrobial effect[25]. Protopine has been reported to have antibacterial activity[26]. Similarly, Corydine has DNA damaging activity[27]. Nakhuru et al.



A B

Figure 8: Chemical structure of the major alkaloids in methanol extract of *Dicentra scandens.* A. Protopine B. Corydine.

(2013) reported that alkaloid extract from the root of *Dicentra scandens* exhibited antimicrobial activity against *Bacillus mycoides*, *Bacillus subtilis*, *Escherichia coli*, *Enterobacter cloacae* and some fungal strains[28]. In the present study, the methanol extract of *Dicentra scandens* (D.Don) Walp. showed antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Shigella flexneri*, *Vibrio cholerae* O139, *Bacillus cereus* and *Staphylococcus aureus* which are associated with various human diseases. The antimicrobial activity in the extract may be related to the presence of high amount of alkaloid along with phenolic compounds in the extract. However, further investigations are required to isolate and characterise the various bioactive components of *Dicentra scandens* responsible for the antimicrobial activity.

CONCLUSION

In this study the methanol extract of the root of *Dicentra scandens* exhibited significantly ($p<0.05$) higher antimicrobial activity against both Gram-positive and Gram-negative test bacteria. The antimicrobial activity of the extract could be due to the presence of the high amount of alkaloids in the extract as detected by the GC-MS analysis. The results of the present study revealed potential antimicrobial activity of *Dicentra scandens* (D.Don) Walp. against the test Gram negative and Gram positive bacteria and thus may provide a scientific rationale for the use of *Dicentra scandens* (D.Don) Walp. in traditional medicine. Hence the root of this plant could be a potential source of natural antimicrobial agent.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS

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