Original Article

Dicentra scandens (D. Don) Walp. Root Phytochemical Constituents Against Potential Wound Pathogens

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ABSTRACT

Objectives: To investigate the phytoconstituents of *D. scandens* and evaluate its activity against potential wound pathogens to validate its uses.
Materials and methods: Antibacterial and antifungal activity of the root extracts against bacterial strains and one clinically important yeast strain was assessed using the agar diffusion assay, minimum inhibitory concentration studies and their effect was compared with some standard antibiotics. The presence of major phytoconstituents was detected qualitatively and quantitatively.
Results: Aqueous ethanol and crude alkaloid extracts showed maximum inhibition of 37.5% and 33.13% and a minimum inhibitory concentration of 100 µg/ml and < 10 µg/ml against *Streptococcus mutans* and *Proteus mirabilis*, respectively. Most of the test pathogens, except *P. mirabilis,* were found to be susceptible to the two standard antibacterial and two standard antifungal drugs assayed. The phytochemicals detected included alkaloids, cardiac glycosides, tannins and terpenoids. Phytochemical analysis showed the presence of ~ 5% alkaloids.
Conclusion*:* The results indicated that the *D. scandens* possesses potential broad-spectrum antimicrobial properties and the phytochemicals detected correlate well with the ethnomedicinal uses and hence require detailed phytochemical investigation for potential pharmaceutical applications.

Keywords: *Dicentra scandens*, phytochemicals, antimicrobial activity, *Proteus mirabilis*, *Pseudomonas aeruginosa, Streptococcus mutans*.

INTRODUCTION

Until the advent of antibiotics in the 1950s, the use of plant derivatives as antimicrobials had been virtually non-existent[1]. Screening of medicinal plants for antimicrobial activity has recently gained importance, as the World Health Organization (WHO) is keenly interested in the development and utilization of traditional medicinal plant resources in developing countries, in order to extend healthcare to the maximum number of people in these countries[2]. According to Iwu *et al*[3], the demand for medicinal plants is increasing as natural products are being recognized as abundantly available, safe and cost-effective alternatives to synthetic medicines and are sometimes the only source of healthcare for the poor. Moreover, mainstream medicine is increasingly receptive to the use of antimicrobials and other drugs derived from plants, as traditional antibiotics become ineffective. Therefore, various researchers[4,5] have the opinion that in view of the ever increasing incidence of resistance to existing therapeutic agents, there is a constant need to develop new and effective antimicrobial drugs for the treatment of infectious diseases from plants. Of the estimated 250,000–400,000 plant species, only 6% have been studied for their biological activity and about 15% investigated phytochemically[6,7]. This shows that plants represent a vast untapped source of medicines, having enormous therapeutic potential not only for treating various diseases, but also for mitigating the side effects that are often associated with synthetic drugs.

Dicentra scandens (D. Don) Walp. has been used traditionally as a remedy for dysentery, diarrhoea, fever, hypertension, diabetes, antihelmentic and wound healing[8,9]. According to Jamir *et al*[10], *D. scandens* leaves are taken raw for asthma problems and the paste of tuberous root is used as antidote for insects and snake bites. Nakhuru *et al*.[11] has reported the antimicrobial activity of this highly valuable medicinal herb. Very less scientific literatures are available on this herb, though some tribal communities of North East India valued it as one of the most potent medicinal herbs amongst the diversified medicinal flora and fauna in this biodiversity hot spot region. The purported medicinal uses of this herb are yet to be scientifically evaluated and validated. Therefore, the objectives of the present investigation were to screen the phytochemicals and evaluate its activity against various human pathogens, including clinically important opportunistic pathogens, thereby validating the claims of *D. scandens*.

MATERIALS & METHODS

Collection of plant material

D. scandens (Fig. 1) was collected during February, 2011 from Mao in Senapati district (93.29°E and 94.15°E and 24.37°N and 25.37°N) of Manipur state, Northeast India. A voucher specimen of the herb was deposited in the herbarium of the Department of Botany, North Eastern Hill University, Shillong (collection no. 160 and accession no. NEHU 11 877).

Chemicals

Amphotericin, cefuroxime, fluconazole, gentamicin, nutrient agar (NA), Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB) and Muller Hinton broth were obtained from Himedia, Mumbai, India. Other reagents and solvents of analytical grade were purchased from Himedia and Merck, Mumbai, India.

Preparation of the plant extracts

Collected root tubers were washed with running tap water, shade-dried for 2

weeks and powdered using a mechanical grinder. n- hexane, ethyl acetate and 80% ethanolic extracts were screened for phytochemicals. About 50 g each of ground sample was soaked in each solvent for 48 h. The extract was filtered using Whatman No.1 filter paper and the filtrate was concentrated under reduced pressure in a rotary vacuum evaporator (RV10 Control, IKA, Germany)[12]. Concentrated extract was air-dried to a constant weight at room temperature and used for preliminary phytochemical screening by standard qualitative procedures[13-16]. The ethanolic extract was used for evaluating antimicrobial activity and crude alkaloid extraction.

Preparation of crude alkaloid extract

The hydroethanolic extract was extracted using a weakly acidic solvent which was further extracted with dichloromethane. The aqueous acidic phase collected was basified using NH_4OH and further extracted with an equal amount of dichloromethane. The dichloromethane phase, rich in alkaloids, was separated, evaporated and weighed[12]. The yield of the alkaloid fraction was about 5% (w/w). The crude alkaloid extract thus obtained was further confirmed by different tests. The resultant crude alkaloid extract was stored at – 20°C for further analyses.

Test extracts preparation

10% (w/v) of extract solutions (aqueous ethanolic and crude alkaloids) were prepared by dissolving in a small amount of dimethyl sulphoxide (DMSO) and the final volume was adjusted with double distilled water. The solutions were sterilized by filtering through a Millipore (0.2 µm) filter and stored.

Test microorganisms

Five pathogenic strains of bacteria and one fungal strain were obtained from the Institute of Microbial Technology, Chandigarh, India. The bacterial strains *Proteus mirabilis* MTCC743, *Pseudomonas aeruginosa* MTCC741, *Streptococcus mutans* MTCC497, *Salmonella enterica typhi* MTCC733, *Escherihcia coli* MTCC40 and yeast, *Candida albicans* MTCC854 were used for the study.

Determination of antimicrobial activity

The antimicrobial potential of the aqueous ethanolic and crude alkaloid extracts was determined by measuring the diameter of the zone of inhibition (ZI) in mm around the well according to the Indian Pharmacopeia Commission, 2007[17]. Percentage inhibition was calculated according to Vyas *et al*.[18] (Table 1). Strain revivified nutrient broth (100 µl) was poured into a sterile petri plate, followed by the addition of 20 ml of slightly warm (60°C) nutrient agar and potato dextrose agar, which were allowed to solidify. On setting, 6-mm wells were bored in the middle of each plate onto which 100 µl of test sample was added and incubated for 24 h at 37 ± 1°C for bacteria and 72 h at 30°C for fungus. Culture broth and 1% DMSO (100 µl each) were inoculated into agar wells as a negative control. The susceptibility of the test pathogenic microorganisms to known antibiotics was also tested as a positive control. Agar dilution method was used to determine the minimum inhibitory concentrations (MICs). Stock solutions were obtained by dissolving test samples in a 1 % DMSO. Serial dilutions were made to obtain different test concentrations from the stock solution. The experiments were performed in triplicate.

RESULT

Phytochemical constituents

D. scandens in the flowering phenological stage is shown in Figure 1. Phytochemical analyses of the three extracts are tabulated in Table 1. Alkaloids were detected in all three extracts. The presence of

alkaloids was further confirmed using reagents such as Dragendorff's reagent, Mayer's reagents and Hager's reagent. Cardiac glycosides and terpenoids were detected in aqueous ethanolic and ethyl acetate extracts, but not in the hexane extract. Likewise, tannins were detected in the aqueous ethanolic extract alone; however, steroids were detected in both ethyl acetate and hexane extracts. Flavonoids and saponins were not detected in any of the three extracts. Preliminary quantitative assessment of the presence of different phytochemicals in the extracts was graded + to +++ based on criteria such as turbidity, precipitation, color intensity and ring formation characterized by each class of compounds.

Antimicrobial activity of the extracts

The inhibition zone size and percentage inhibition ranged from 11.32 to 30 mm corresponding to 14.15% to 37.5% and 13.5 to 26.5 mm corresponding to 16.87% to 33.13% (Tables 2 and 3). Both the aqueous ethanolic and crude alkaloid extracts showed strong inhibition against *S. mutans* with zones of inhibition of 30 and 26.5 mm, respectively. The extracts exhibited growth inhibition of all the pathogenic strains tested (Tables 2 and 3).

MICs of the extracts

Tables 2 and 3 show the overall activities of ethanolic and crude alkaloid extracts against tested human pathogens. Ethanolic and crude alkaloid extracts had excellent activities against *P. mirabilis*. Concentrations of 100 µg/ml of ethanolic and < 10 µg/ml of crude alkaloid extracts were found to inhibit *P. mirabilis*. Comparatively lower concentrations were found to inhibit Gram-positive strains than the Gram-negative strains and yeast.

Antibiotic susceptibility profile of test pathogens

Four antibiotics were used as positive control against the susceptibility of the test pathogens. Gentamicin and cefuroxime were found to be effective against the test, Gram-positive and Gram-negative pathogenic bacteria and amphotericin and fluconazole against *C. albicans*. However, *P. mirabilis* was found to be resistant to cefuroxime, whereas the other test pathogens were sensitive to the test antibiotics (Table 4).

DISCUSSION

The active components of many drugs of plant origin are secondary metabolites[19]. Therefore, analysis of plant extracts for their main bioactive phytocompound(s) is vital. Phytochemical analysis revealed the presence of alkaloids, tannins, terpenoids and cardiac glycosides in the extracts of *D. scandens*. Phytochemicals such as phenolics, polyphenols, alkaloids, terpenoids, etc., are known to possess antimicrobial activity. Good amount of terpenoids were detected in the aqueous ethanolic extract and the activity of which are well documented against bacteria[20-22] and fungi[23,24]. The mechanism of action of terpenes is speculated to be membrane disruption by lipophilic compounds. Alkaloids were detected in significant amounts in all the three extracts. However, the most abundant amount of alkaloids was detected in the aqueous ethanolic extract. Therefore, aqueous ethanolic extract was processed further for crude alkaloids and evaluation of its effect against potential wound pathogens and encouraging results obtained. It is important to remember here that different microorganisms can exist in polymicrobial communities and this is often the case within the margins of wounds that delay the healing process. Therefore, it is important to control the growth of wound flora. The antimicrobial properties of alkaloids have been well established and

Verpoorte[25] has reported as many as 300 alkaloids showing such activity. Further fractionation of the crude alkaloid extract may lead to novel alkaloids. *P. aeruginosa*, an opportunistic pathogen, causes serious wound infections and colonizes rapidly, and is then disseminated quickly from wounds into the bloodstream, which is often fatal. Moreover, one of the major problems associated with infection by this pathogen is its resistance to most conventional antibiotics[26,27]. When the crude alkaloid extract was bio-assayed against *P. aeruginosa,* the inhibitory activity was 24.38% compared with 14.15% inhibition by the extract, though the MICs of both extracts remained the same. The observed antimicrobial activity in this study established the scientific rationale for its ethnomedicinal use in wound healing[8]. Furthermore, this finding sheds some light on a possible source for treatment of wounds due to such a clinically important opportunistic pathogen. *C. albicans* was inhibited both by aqueous ethanolic and crude alkaloid[11] extracts of *D. scandens.* Activity of medicinal plant extract against opportunistic human pathogens like *C. albicans* is clinically relevant as it is listed among the top opportunistic pathogens of immune-compromised hosts such as AIDS patients. *S. mutans* was found to be highly susceptible to the extracts as well as the drugs gentamicin and cefuroxime in our study. Thus, this result supports the use of this herb for relieving tooth ache[9] as *S. mutans* is one of the causal agents in tooth caries. Activity against causal agents of diarrhoea and dysentery such as *S. enterica typhii*, *E. coli*[11] and *P. mirabilis* by the extracts correlates well with the claims of *D. scandens* for gastrointestinal disorders. The majority of *Proteus* infections in humans is caused by *P. mirabilis*. Cefuroxime, a second generation cephalosporin with increased resistance to β-lactamase hydrolysis, is reported to be highly effective (about 90% inhibition at 12.5 µg/ml) against *P. mirabilis*[28]. Thus, cefuroxime was used as a reference drug to compare its efficacy with the test extracts. Although other bacteria were sensitive to cefuroxime, *P. mirabilis* was resistant, which is in contrast with the earlier report[28]. However, test extracts were observed to be highly active against *P. mirabilis*. Among the test pathogens, *P. mirabilis* exhibited the highest susceptibility to test extracts in terms of MICs thereby the ethnomedicinal uses of this herb against gastrointestinal disorders is justified by the outcome of the investigation. As *P. mirabilis* was found most sensitive to alkaloid extract in the study, it implies that some component in the alkaloid fraction may be responsible for the activity. However, the same extract was not so efficacious against *S. enteric typhii* and *E. coli*. This difference could be attributed to the presence of extra outer membranes in their cell wall acting as a barrier for the compound(s) to diffuse into the bacterial cells. The activity may be contributed partly by tannins as tannins are well documented to be anti-diarrhoea and anti-haemorrhagic[29] which is in agreement with an earlier report that the decoction of this plant is used as a remedy against diarrhoea and dysentery[30,31]. The activity of the reference drugs was similar or comparatively higher than that of test extracts (Table 4), except against *P. mirabilis* (which was resistant to cefuroxime), and the alkaloid extract showed slightly higher activity against *P. aeruginosa and S. enterica typhii* than that of the cefuroxime. Thus, the antibacterial and antifungal activity against potential wound pathogens observed in the investigation could be attributed to the different phytochemicals present in this plant.

CONCLUSION

D. scandens may be added to the growing list of plants with potential biological activities. The results of the present investigation showed that the aqueous ethanolic and crude alkaloid extracts are

broad-spectrum agents, exhibiting pronounced antibacterial and antifungal activity against most of the tested pathogens. This antibacterial and antifungal activity exhibited by the plant may be attributed to the various phytochemicals, such as alkaloids, terpenoids and tannins present in the extracts. Detailed phytochemical investigation may be taken up for potential pharmaceutical applications.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1. Phytochemical constituents of *Dicentra scandens* tuberous root extracts

Phytoconstituents	Hydroethanolic	Ethyl acetate	N-hexane	Test
Flavonoids	-	-	-	HCl and Mg ribbon
Alkaloids	+++	++	++	Alkaloid reagents used: Mayer's, Dragendrorff's Hager's (Picric acid)
Saponins	-	-	-	Frothing in aqueous
Tannins	+	-	-	5% $FeCl_3$
Steroids	-	+	+	Liebermann-Buchard
Terpenoids	++	+	-	Keller-Keliani
Cardiac glycosides	+++	++	-	Salkowski

Key: - : Not detected; +: in trace; ++: light; +++: heavy

Table 2. Antimicrobial activity and minimum inhibitory concentrations of ethanolic extract of *Dicentra scandens* tuberous root

Test microorganisms	MTCC Accession no.	Zone of inhibition [mm]	Inhibition [%]	MICs [µg/ml]
Escherichia coli	MTCC 40	20.30	25.37	5000
Proteus mirabilis	MTCC748	15.03	18.85	100
Pseudomonas aeruginosa	MTCC741	11.32	14.15	1000
Streptococcus mutans	MTCC497	30.00	37.50	500
Salmonella enterica typhii	MTCC733	14.04	17.55	5000
Candida albicans	MTCC854	14.75	18.43	5000

Values are represented as mean of 3 replicates

Table 3. Antimicrobial activity and minimum inhibitory concentrations of crude alkaloid extract of *Dicentra scandens* tuberous root

Test microorganisms	MTCC Accession no.	Zone of inhibition [mm]	Inhibition [%]	MICs [µg/ml]
Escherichia coli	MTCC 40	-	-	4000
Proteus mirabilis	MTCC748	13.5	16.87	<10
Pseudomonas aeruginosa	MTCC741	19.5	24.38	1000
Streptococcus mutans	MTCC497	26.5	33.13	1000
Salmonella enterica typhii	MTCC733	21.5	26.88	5000
Candida albicans	MTCC854	-	-	5000

Values are represented as mean of 3 replicates, -: not done

Table 4. Susceptibility profile of test human pathogenic microorganisms against known antibiotics

Antibiotics (µg)	Diameter of zone of inhibition in mm							
	Bacteria							Yeast
	Gram (-)					Gram (+)		
	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*	*S. enteric typhii*	*S. aureus*	*S. mutans*	*C. albicans*
Gentamicin (10)	35.45	18.03	30.90	31.23	33.07	22.56	33.56	-
Cefuroxime (30)	30.09	18.98	R	18.27	19.07	20.67	40.45	-
Amphotericin (30)	-	-	-	-	-	-	-	13.04
Fluconazole (10)	-	-	-	-	-	-	-	35.78

Values are represented as mean of 3 replicates. R: resistant, -: not applicable

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Figure 1. *Dicentra scandens* during phonological stage