



PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF CLEMATIS GOURIANA ROXB.

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Date Received:

4th Apr 2014

Date of Accepted:

14th Apr 2014

Date Published:

12th May 2014

Abstract:

The water, ethanol and petroleum ether extracts of *Clematis gouriana* was assessed for their antibacterial properties against 3 Gram positive (*Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*) and 4 Gram negative (*Vibrio cholerae*, *Escherichia coli*, *Shigella dysenteriae* and *Shigella flexneri*) bacteria. The aqueous and ethanol extracts of different parts of the plant showed considerable antibacterial activities against all the bacteria tested except *Vibrio cholerae* and *Shigella flexneri*. However, the petroleum ether extract of different parts of the plant showed antibacterial activity against all the bacteria except *Vibrio cholerae*. Among all the solvents, the petroleum ether extract showed highest antibacterial activity indicating the release of most of the bioactive substances dissolved in the solvent. Further, the leaf extract of all the solvents showed maximum antibacterial activity. The inhibition of both Gram positive and Gram negative bacteria indicated the presence of broad spectrum antimicrobial substances in the plant.

Keywords: Phytochemicals, *Clematis gouriana*, Antibacterial activity

Introduction

Since the early ages of civilization phytomedicines have been used as the mainstay in the treatment of various diseases and preservation of human health. Farnsworth et al (1985) ^[1] reported that 80% of the population in the developing world still rely on traditional medicine for their primary health care needs. Unfortunately, most of the herbal medicinal remedies have not been assessed for their safety as a traditional medicine practitioners believe that herbs are safe simply because they are natural in origin. The genus *Clematis* (Ranunculaceae) has about 300 species. The roots and rhizomes of *Clematis* are

traditionally used as an analgesic, abirritative, antibacterial, antiphlogistic, anticancer and diuretic agent. In folk medicine, it is used for the treatment of blisters and as a poultice for festering wounds and ulcers ^[2]. Despite the various uses of different *Clematis* species in traditional medicine, there is inadequate information about the antimicrobial activity of this plant ^[3,4]. However, this plant has not been scientifically validated for its antibacterial activity. Hence the present study was initiated to determine the antibacterial potential of *Clematis gouriana* against different pathogenic bacteria.

MATERIALS AND METHODS

Plant material collection

Clematis gouriana leaves and stems, collected in April, 2009 from a natural population growing in Nainital Hills, Nainital, Uttarakhand, was identified by Prof S. K. Mukherjee, Plant taxonomist, Botany Department, University of Kalyani. A voucher specimen (SNSCG2) was deposited at the herbarium of the University.

Since the same species in different ecological locations may not have the same antibiotic or antimicrobial activity, information regarding time and place of collection, its state of maturity, habitat and altitude were recorded.

Preparation of plant extracts

The plants (leaf and stem) were shade-dried at ambient temperature (30°C) and were powdered using an electronic blender. Then the aqueous extract was prepared by suspending 100g of dried powdered in 600 ml of sterilized double distilled water. Subsequently, solvent extracts were prepared by soaking the powdered material in 600ml of each of the solvents viz., ethanol and petroleum ether in a soxhlet apparatus for 72h at 300C until complete extraction.

Test bacteria

The bacterial strains used to test the antibacterial potential were *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Vibrio cholerae*, *Shigella dysenteriae*, *Shigella flexneri* and *Pseudomonas aeruginosa*.

Phytochemical screening

The initial phytochemical screening of different solvent extract was performed following standard method [5].

Antibacterial activity

The antibacterial efficacy of different extracts of various parts of the plant was studied by agar well diffusion method [6].

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of ethanolic extracts showed the presence of various types of active components namely alkaloids, saponins, tannins, flavonoids, terpenoids and steroids. The petroleum ether extracts, however, showed the presence of these phytochemicals in higher amount (Table 1).

Antibacterial activity

Table 2 showed the antibacterial activities of aqueous extracts of *Clematis gouriana* against three Gram

positive and five Gram negative bacteria. The extract showed antibacterial activity against all the bacteria except *Vibrio cholerae* and *Shigella flexneri*. The leaf extract showed somewhat better activity than that of stem extract. The leaf extract showed highest antibacterial activity against *Micrococcus luteus* and least antibacterial activity against *Shigella dysenteriae* while the stem extract exhibited highest activity against *Staphylococcus aureus* and least activity against *Shigella dysenteriae*.

The crude ethanolic leaf extract of *Clematis gouriana* showed highest antibacterial activity (18mm) against *Micrococcus luteus* and least antibacterial activity (12mm) against *Shigella dysenteriae* (Table4). Comparatively, somewhat lower activity was observed in case of ethanolic stem extract. Here also highest activity was noted against *Staphylococcus aureus* and *Micrococcus luteus* with lowest activity against *Shigella dysenteriae*. *Vibrio cholerae* and *Shigella flexneri* were found to be insensitive to both ethanolic leaf and stem extract (Table 4).

The crude petroleum ether extract of leaf of *Clematis gouriana* exhibited strong inhibitory effect against *Staphylococcus aureus* and weak inhibitory effect against *Shigella flexneri*. Similar results were also obtained from the stem extract (Table5). *Vibrio cholerae* was found to be insensitive to both the petroleum ether extract of leaf and stem.

CONCLUSION

Aqueous and solvent (ethanol and petroleum ether) extracts of *Clematis gouriana* were examined for their antibacterial potential against some reference strains of human pathogenic bacteria. Different parts (leaf and stem) of the plant studied showed considerable antibacterial properties against all bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Vibrio cholerae*, *Escherichia coli*, *Shigella dysenteriae* and *Shigella flexneri*). Among all the solvents the petroleum ether extracts showed maximum antibacterial activity, which indicated the release of most of the bioactive substances in the solvent. Moreover, the leaf extract of all the solvents of this plant were found to be more potent in suppressing the bacterial activity. The inhibition of both Gram positive and Gram negative bacteria indicated the presence of broad spectrum antimicrobial substances in the plant. However, Gram positive bacteria were found to be very sensitive than Gram negative one. In case of all extracts, *Vibrio cholerae* was found to be insensitive. *Shigella dysenteriae* and *S. flexneri* were insensitive to aqueous and ethanol extracts of both leaf and stem of the plant. The results provide a scientific basis for the centuries old usage of aqueous and solvent extracts of this plant.

Table 1: Qualitative phytochemical screening of different parts of *Clematis gouriana*

Phytochemicals	Leaf	Stem
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Anthraquinones	+	+
Steroids	+	+
Flavonoids	+	+
Phenols	-	-
Terpenoids	+	+

+ indicates presence or positive reactions, - indicates absence or negative reaction

Table 2: Antibacterial activity of different parts of *Clematis gouriana* water extract

Plant parts	Zone of inhibition (mm)					
	Bs	Sa	MI	Ec	Vc	Sd
Leaf	12	15	16	12	nd	10
Stem	11	13	12	10	nd	8

Bs *Bacillus subtilis*, Sa *Staphylococcus aureus*, MI *Micrococcus luteus*, Ec *Escherichia coli*, Vc *Vibrio cholerae*, Sd *Shigella dysenteriae*, Sf *Shigella flexneri*, nd not detected

Table 3: Antibacterial activity of different parts of *Clematis gouriana* ethanol extract

Plant parts	Zone of Inhibition (mm)					
	Bs	Sa	MI	Ec	Vc	Sd
Leaf	14	17	18	14	nd	12
Stem	12	14	14	12	nd	10

Bs *Bacillus subtilis*, Sa *Staphylococcus aureus*, MI *Micrococcus luteus*, Ec *Escherichia coli*, Vc *Vibrio cholerae*, Sd *Shigella dysenteriae*, Sf *Shigella flexneri*, nd not detected

Table 4: Antibacterial activity of different parts of *Clematis gouriana* petroleum ether extract

Plant parts	Zone of Inhibition (mm)					
	Bs	Sa	MI	Ec	Vc	Sd
Leaf	20	22	20	15	nd	14
Stem	17	17	16	14	nd	12

Bs *Bacillus subtilis*, Sa *Staphylococcus aureus*, MI *Micrococcus luteus*, Ec *Escherichia coli*, Vc *Vibrio cholerae*, Sd *Shigella dysenteriae*, Sf *Shigella flexneri*, nd not detected

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