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ARTICLE

METHANOLIC EXTRACTS OF *CLEMATIS TERNIFLORA* LEAF AND STEM AS POTENTIAL ANTIMICROBIAL AGENTS

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Abstract

Clematis terniflora belonging to the family Ranunculaceae is native to China and Japan where in India it is considered as an ornamental plant. It is used as tribal medicine and also used in the treatment of various ailments such as nervous disorders, syphilis, gout, malaria, dysentery, rheumatism, asthma etc. In the present study methanolic extract of *Clematis terniflora* leaf and stem were subjected to antibacterial and antifungal properties. Human pathogenic bacteria *Bacillus substilis, Pseudomonas fluorescence, Staphylococcus aureus, Escherichia coli* were used to evaluvate antibacterial property while *Candida albicans, Aspergillus niger, Fusarium solani, Pencillium notatum* were used to evaluate the antifungal properties. The extract showed highest antibacterial activity against *Escherichia coli.* Antifungal screening of these plant extract showed highest activity in *Fusarium solani, Pencillium notatum* and *Candida albicans*. Overall results suggest that the plant extract have great potential as an antimicrobial agent against certain fungi and bacteria.

Keywords: Ranunculaceae, *Candida albicans, Staphylococcus aureus*, Potato dextrose agar (PDA).

Introduction

From ancient period, plants were considered as a fundamental source of potent drugs in traditional medicine. In recent years there is an increasing trend of intensive studies on extracts from plants to investigate the new antimicrobial agent for natural therapies. Plants synthesize bioactive substances in the form of secondary metabolites which may be responsible for antimicrobial activity. The antimicrobial property of most plants makes them to be valuable in the ethno medicine. Antibiotic resistant strains of clinically important pathogens has drawn global attention which has urged scientists to search for potential antimicrobial herbal medicine(Aibinu et al.,2003). Thus, the effectiveness of plant extracts on microorganism has been studied worldwide that can serve as source for the new antimicrobial drugs(Pretorius et al.,2003). *Clematis terniflora* DC was selected for the present study. It is a climber native from China and Japan. *Clematis terniflora* commonly known as sweet autumn. Traditionally it is used for treating nervous disorders, syphilis, gout, malaria, dysentery, rheumatism, asthma etc. The present study focussed on the anti microbial activity of the plant. Anti microbial activity includes antibacterial and antifungal properties.

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Materials and Methods

Collection of plant material:

Fresh plant materials were collected from various gardens of Thiruvananthapuram.

Preparation of the plant extract:

The fresh leaves and stem of *C. terniflora* were collected, washed and shade dried at room temperature. The dried leaves and stem were powdered using a mechanical grinder and stored in air tight containers for further use. 10gm each of the leaf and stem powder were subjected to Soxhlet extraction using 200ml methanol. The extract were concentrated under reduced pressure in a rotary evaporator (Superfit, Rotavap) and stored in the refrigerator till further use. The extract was weighed and made up 100 mg/ml and a known volume was used for antimicrobial assay.

Eight important strains of micro organisms were selected for the present study which included bacteria (*Bacillus substilis, Pseudomonas fluorescence, Staphylococcus aureus, Escherichia coli) and fungi (Candida albicans, Aspergillus niger, Fusarium solani, Pencillium notatum*).

Antibacterial assay:

Agar well diffusion method (Smania et al.,1999; 2007) is widely used to evaluate the antimicrobial activity of the compound. Autoclaved 15-20 mL of Mueller-Hinton agar was poured on glass petri plates and allowed to solidify. Standardized inoculum of the test organism was uniformly spread on the surface of these plates using sterile cotton swab. Four wells with a diameter of 8 mm (20 mm apart

from one another) were punched aseptically with a sterile cork borer in each plate. Compound solution (40 and 80 µL) at desired concentration from 100mg/mL stock was added to three of the wells and one well with Gentamycin as positive and compound solvent as negative control. Then, the agar plates were incubated under 37°C for 24 hrs. After incubation, clear zone was observed. Inhibition of the bacterial growth was measured in mm

Antifungal assay:

Antimicrobial susceptibility testing was done using the well diffusion assay to analyse the anti-fungal activities of the plant extract. A sterile swab was used to evenly distribute fungal culture over the PDA agar medium. The plates were allowed to dry for 15 minutes before use. Desired concentration of the compound (40 and 80 µL) from 100 mg/mL stock was added to the wells. One well with cotrimazole was taken as the positive control and solvent without the compound as negative control. The plates were incubated at room temperature for 3 days after which they were examined for inhibition zones.

Table 1. Distribution of different repeat type classes of SSR

SL.No	Sample	Organism	Diameter of zone (mm)		
			+	T1	T2
1	CL	*Bacillus substilis*	40	-	-
2	CS	*Bacillus substilis*	45	-	-
3	CL	*Pseudomonas fluorescence*	22	-	-
4	CS	*Pseudomonas fluorescence*	21	-	-
5	CL	*Staphylococcus aureus*	32	2	5
6	CS	*Staphylococcus aureus*	30	1	4
7	CL	*Escherichia coli*	29	-	11
8	CS	*Escherichia coli*	35	-	-

Fig 1: Antibacterial activity in *Clematis terniflora*. 1; leaf extraction inhibit against *Staphylococcus aureus*, 2; leaf extraction inhibit against *Escherichia coli*.

Results and Discussion

Anti bacterial activity: The methanolic leaf and stem extracts of *Clematis terniflora* displayed less antibacterial activity compared to standard antibiotic agent against gram positive and gram negative bacteria (Table 1 & fig 1,2). The extraction showed an inhibition zone ranging from 1 to 5 mm respectively against Staphylococcus aureus. Staphylococcus can cause wide variety of diseases in humans and animals through either toxin production or penetration. Methanolic *E. coli* strains do not cause disease but virulent strains can cause gastroenteritis, urinary tract infections and neonatal meningitis. The leaf and stem extraction showed no inhibitory activity against *Bacillus substilis* and Pseudomonas fluorescence. Similarities can be observed in the results of study that the ethanolic extract of *Clematis papuasica* against bacteria such as Staphylococcus and *E. coli* had considerably antimicrobial effects, but Pseudomonas was not sensitive to these extracts (Khan et al.,2001).

Anti fungal activity:

Antifungal activity of methanolic leaf extract showed the inhibitory zone of 10mm and 12mm against Candida albicans. Candida is a genus of yeast and is the most common cause of fungal infections worldwide (Manolakaki et al., 2010). Previously studies on *Clematis hirsuta* (leaves) reported that high antifungal activity against *Candida albicans* (Cos et al., 2002). It can cause infection in humans and animals. There is no inhibitory activity for stem extract against *Candida albicans*. Methanolic stem extract (80 µl) showed an inhibitory zone of 2mm against Asperigillus niger. Some species cause infections in humans and animals. There was no activity for stem (40 µl) and leaf (40 µl, 80 µl) extracts. Methanolic leaf extracts of concentrations 40 µl and 80 µl showed an inhibitory zone of 15mm and 29 mm against *Fusarium solani*. In the present study 80µl concentration of leaf extract showed more activity than standard antibiotic. There is no activity for stem extract against *Fusarium solani*. The methanolic extraction of leaf (40µl and80µl) showed an inhibitory zone of 22mm and 30mm against *Pencillium notatum*. There is no activity for stem extract against the fungus. In the present study

Table 2. Anti fungal activity of *Clematis terniflora*

Sl.No	Sample	Organism	Diameter of zone (mm)		
			+	T1	T2
1	CS	*Candida albicans*	20	-	-
2	CL	*Candida albicans*	20	10	12
3	CS	*Asperigillus niger*	30	-	2
4	CL	*Asperigillus niger*	30	-	-
5	CS	*Fusarium solani*	20	-	-
6	CL	*Fusarium solani*	21	15	29
7	CS	*Pencillium notatum*	30	-	-
8	CL	*Pencillium notatum*	30	22	30

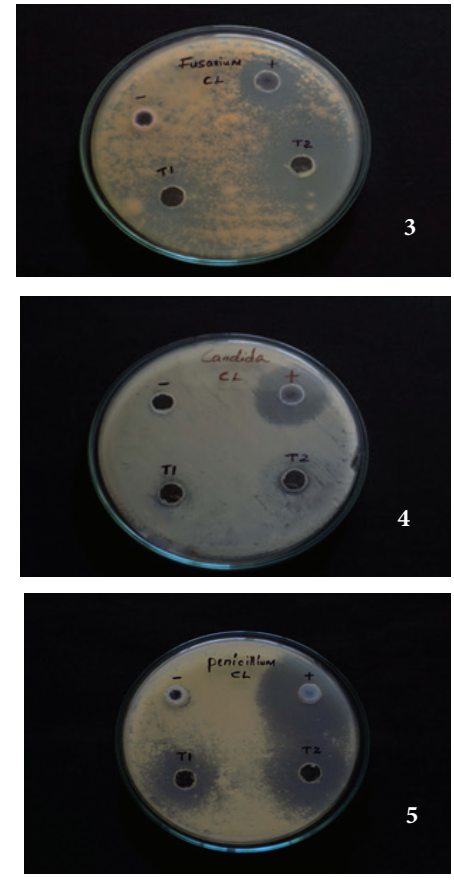

Fig 2: Anti fungal activity of *Clematis terniflora*. 3: leaf extraction inhibit against Fusarium solani, 4: leaf extraction inhibit against Candida albicans, 5: leaf extract inhibit against Pencillium notatum

methanolic extraction of leaf were found to have more antifungal activity against certain fungi, *Fusarium solani, Pencillium notatum* and *Candida albicans*. It has been reported that, methanolic extract of *Clematis vitalba* also shows similar effects (Buzzini et al., 2003). The antifungal

Conclusion

The two concentrations (40µl & 80µl) of methanolic extracts of leaf and stem were used for the anti bacterial and antifungal analysis. Anti bacterial activity is less compared to the standard antibiotic available in the market. Methanolic leaf extraction (80 µl) showed greater inhibitory zone against Ecoli compared to other organism. The stem extraction showed less anti fungal activity. The leaf extraction have high inhibition against *Fusarium solani,* Pencillium and *Candida albicans*. The results suggest that this plant might be a candidate against certain fungi and bacteria, especially showing higher activity against fungi.

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