



Original Research Article

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Phytochemical Analysis and Antimicrobial Activity of Different Extracts of *Clematis gouriana*. Roxb Flower on Multi Drug Resistance oral Pathogens

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Different extract of *Clematis gouriana* Roxb flower was assessed for its phyto constituents and *in vitro* antimicrobial activity against multidrug resistance (MDR) oral pathaogens. Phytochemical analysis revealed the presence of various important secondary metabolites like alkaloids, flavonoids, phenol, emodins, coumarins etc. Among the different extracts of *Clematis gouriana* Roxb flower, ethanol extracts was found to be more potent against all the MDR oral pathogens tested, significant activity was seen against *E. coli* with the zone of inhibition of 13mm and the MIC value of 0.875mg/ml, followed by against both *S. aureus* and *S. mutants* (12 mm) with MIC value of 0. 1.75mg/ml and 0.875mg/ml respectively.

Introduction

Medicinal plants are being used for the treatment of various diseases from the pre historic period in the traditional systems of medicine which is still in practice. With the progress in scientific research of herbals, medicinal herbs are considered to have a pivotal role in this synthetic era. These medicinal plants find wide application in pharmaceutical, cosmetic and food industries.

As the herbal products are safer in contrast to the synthetic, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased demand on the use of plant materials as a source of medicine for various human ailments. Most of the human pathogens have developed resistant against many commonly used synthetic antibiotics in the treatment of various infections and communicable

diseases which has created alarming clinical situations in the treatment of infections. Many reports show that gram-negative bacteria have a higher resistance to plant extracts than gram-positive bacteria. This may be due to the difference in their cell wall structures (Paz *et al.*, 1995; Vlietinck *et al.*, 1995; Kudi *et al.*, 1999; Palambo and Semple, 2001).

Hence it has become necessary for the search of new antimicrobial agent from other sources, the most convenient natural source is the plants as they produce a variety of compounds to protect themselves against various pathogens, may be because of this reason in traditional medicine plants are being used to treat chronic as well as acute infectious diseases.

Medicinal plants have been used for its antibacterial, antifungal and antiviral activities from many decades in different parts of the world (Ali *et al.*, 1998; Barbour *et al.*, 2004; Yasunaka *et al.*, 2005). The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have significant value in treating resistant microorganism strains (Eloff, 1999).

Clematis gouriana Roxb., is a woody climber belongs to the family Ranunculaceae. It is widely used in Ayurveda for the treatment of various ailments. Whole plant is poisonous, stem and leaves are bitter, the juice of freshly crushed leaves and stems has a vesicant blistering action.

Whole plant juice is used is to treat cold, headache, for wound healing, infections. Leaf extract is used to treat eczema, boils, itches, also applied to scabies, cuts and wounds, powdered leaves are

used as snuff for sinusitis.. Flowers are used as insecticides. Roots decoction is given to treat stomachache (CRC World Dictionary of Medicinal and Poisonous Plants). Aerial parts and roots contain a quaternary aporphine alkaloid, magnofluorine. The leaves yield protoanemonin, a fungitoxic compound. Some researchers have proved its efficacy as antimicrobial agents (Sankar Narayan Sinha 2006), anti ulcerative and hepato protective activity (Kulkarni A. S *et al* 2012), anti inflammatory activity (Gulnaz.A.R *et al* 2014). In the present study different extract of *Clematis gouriana Roxb* flower was assessed for its phyto constituents and *in vitro* antimicrobial activity against MDR oral pathogens to provide scientific validation to this plant.

Materials and Methods

Plant Collection

The Healthy disease free plant *Clematis gouriana Roxb* was collected, from Western Ghats. The plant was identified and authenticated at National Ayurveda Dietetics Research Institute Bangalore, (voucher no: RRCBI- 1194). The flowers were separated from the plants, washed, shade dried at room temperature and then homogenized to fine powder of a 30 mesh sizes and stored in airtight bottles at 4°C.

Extraction of Plant Material

About 50gm of dried flower powder was subjected to extraction by a hot percolation method with different solvent in their increasing polarity (Chloroform, ethyl acetate, ethanol water respectively),in Soxhlet apparatus. Extraction step was carried out until the extractive becomes colorless, the extract was concentrated by using flash evaporator and the dried extracts were stored at 4°C for further study.

Phytochemical Screening

The secondary metabolites like, alkaloids, flavonoids, phenols, tannins, etc were assessed as per the standard procedures. (Gulnaz.A.R and Savitha.G. 2013)

Antibacterial Assay

Microorganisms Tested

Oral pathogenic organisms were isolated from clinical samples (Department of Microbiology, Farooqia Dental College and Hospital Mysore, India). Four multi drug resistant oral pathogens *S. aureus*, *E. coli*, *Lacto bacillus*, and *E.fecalis*, are used in this study.

Antimicrobial Activity by disc Diffusion Method

The disc diffusion assay methods was used to determine antimicrobial efficacy briefly, the inoculums of microorganisms was adjusted to 0.5 McFarland standard, diluted bacterial culture (100 µl) was spread over nutrient agar plates with a sterile swab. 100 µl of the each extracts were applied to each filter paper disc (Whatman No. 1, 6 mm diameter.) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate and the plates were inoculated at 37°C for 24 hrs. Antimicrobial activities were evaluated by measuring inhibition zone diameters (NCCLS).

MIC by 96 well Resazurin based Microtiter Dilution

Resazurin based micro titer dilution assay was used to determine MIC. Under aseptic conditions the first row of micro titer plate was filled with 50 µl of test materials in 10% (v/v) DMSO. All the wells of micro titer plates were filled with 50 µl of nutrient broth. Two fold serial dilution (through out

the column) was achieved by starting transferring 50 µl test material/standard antibiotic from first row to the subsequent wells in the next row of the same column and so that each well has 50 µl of test material/ standard antibiotic in serially descending concentrations. 15 µl of Resazurin solution (as indicator) was added in each well. Finally the inoculums of microorganisms were adjusted to 0.5 Mc Farland standards a volume of 15 µl of the bacterial and fungal suspension added. Each plate was wrapped loosely with cling film to avoid the dehydration of bacterial culture. The plates were incubated in temperature-controlled incubator at 37° C for 24 hrs. The color change in the well was observed visually. Any color change observed from purple to pink or colorless was taken as positive. The lowest concentration of plant leaf extract at which color change occurred was recorded as the MIC value. (Gulnaz.A.R and Savitha.G 2013.)

Statistical Analysis

The result were express as Mean ± SEM. Statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests.

Results and Discussion

Medicinal plants are potential sources to cure various diseases. In contrast to synthetic pharmaceuticals phyto medicines show their curative effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. This synergistic or additive pharmacological effect can be beneficial by eliminating the problematic side effects (Raja and Pugalendi, 2010). The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties (Ljubuncic *et al.*, 2006).

The preliminary phytochemical screening of the present study revealed the presence of

many important compounds which are presented in table-1.

Table.1 Phytochemical Analysis of Ethanolic Extract of *Clematis gouriana* Roxb Flower

Phyto component	CGF C	CGF EA	CGF E	CGF W
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	-	+	+	-
Phenol	-	+	+	+
Coumarin	-	+	+	-
Emodin	-	+	+	+
Steroid	-	+	+	-

Table.2 *In vitro* Antibacterial Activity (Inhibitory Zone in Millimeter) of Different Extracts of *Clematis gouriana*. Roxb Flower against MDR Oral Pathogens

Antimicrobial disc	<i>S. aureus</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>S. mutants</i>
		Zone of inhibition/ mm		
Amoxicillin-Clavulanic acid 30	R	R	R	R
Cefixime	R	R	R	R
Amikacin	7±0.15	8±3.10	7±4.50	9±7.0
Tetracyclin	07±0.10	07±8.25	R	R
Doxycycline	08±5.18	08±0.11	R	07±0.46
chlorhexidine	07±4.10	08±0.46 ^c	07±0.1	07±0.1
CGF C	08±0.80	07±7.10	07±0.0	07±0.4
CGF EA	07±7.10	07±4.15	08±2.38 ^c	09±0.4 ^b
CGF E	12±0.31 ^b	13±0.3 ^a	11±0.4 ^b	12±0.0 ^a
CGF W	09±0.60 ^b	08±0.27 ^c	09±1.10 ^b	10±6.0 ^b

Table.3 Minimum Inhibitory Concentration (MIC) of Different Extracts of *Clematis Gouriana*. Roxb Flower by Resazurin Micro Titre-plate Assay

Antimicrobial source	<i>S. aureus</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>S. mutants</i>
	(MIC, mg/ml)			
Amoxicillin-Clavulanic acid 30	-	-	-	-
Cefixime	-	-	-	-
Amikacin	-	-	-	-
Tetracyclin	5.0±0.50	5.0±0.7	-	-
Doxycycline	2.5±1.25 ^c	5.0±0.57	-	5.0±4.0
Chlorhexidine	5.0±3.40	-	5.0±0.38	5.0±8.5
CGF C	1.75±0.37 ^a	5.0±4.50	5.0±0.45	-
CGF EA	5.0±0.00	-	5.0±5.05	2.5±7.9
CGF E	1.75±0.7 ^a	0.875±2.0 ^a	2.5±8.0 ^b	0.875±0.6 ^a
CGF W	5.0±3.0	-	2.5±0.0 ^c	2.5±0.35 ^c

(+: Present, - : Absent R: Resistant / No activity, CGF C: Chloroform extract, CGF EA: Ethyl acetate extract, CGF E: ethanol extract, CGF W: water extract.). The values are means of triplicates ± standard deviation, the values followed by different superscript differ significantly ^ap<0.001, ^bp <0.01, ^cp<0.05

All the phyto constituents tested were found in the ethanol and ethyl acetate extract. In water extract tannins, coumarins, steroid was absent. In Chloroform extract only alkaloids and flavonoids were detected.

Antibacterial Activity

In the present study, we have evaluated the potential of different extracts of *Clematis gouriana* Roxb flower for its antimicrobial activity against MDR oral pathogens in terms of zone of inhibition and MIC values. The results are presented in table: (2-3). Potent activity was seen in ethanol extract against *E. coli* with the zone of inhibition of 13mm and the MIC value of 0.875mg/ml followed by against both *S. aureus* and *S. mutants* (12 mm) with MIC value of 0. 1.75mg/ml and 0.875mg/ml respectively, Antimicrobial activity was not found with Amoxicillin-Clavulanic acid 30 and Cefixime standard antibiotics against all the MDR oral pathogens. Both *E. fecalis* and *S. mutants* were resistance to the standard antibiotics Tetracyclin, *E. fecalis* was resistance to the standard antibiotics Doxycycline also.

In conclusion, the phyto chemicals/secondary metabolites possess good, antioxidant activities and has been reported to exhibit various biological activities such as antimicrobial, anthelmintic, anti-inflammatory etc. Several researchers have also screened many Indian medicinal plants for their phytchemicals (Jain *et al.*, 2010, Karthishwaran *et al.*, 2010).

The antimicrobial activity is contributed by essential oils, flavonoids, triterpenoids and other natural poly phenolic compounds found in the plants (Ramkumar *et al* 2007). The result of present investigation clearly indicates that the antibacterial activity varies with the solvent and the potent antimicrobial

activity of ethanol against MDR oral pathogens may be due presence of various phyto constituents.

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