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RESEARCH PAPER

Screening of Various Solvent Extracts of *Gymnema sylvestre* R.Br. Leaf for Antibacterial Activity

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ABSTRACT

Increasing emergence of resistance to the currently available antibiotics has necessitated continued search for new antimicrobial compounds. The present study is aimed to confirm ethno- medicinal claim of Gymnema sylvestre leaf possessing antibacterial activity that could be a better alternative for synthetic antibacterial agents, if proved to be effective enough .For this, the antibacterial properties of Gymnema sylvestre leaf were tested against three Gram positive (Bacillus subtilis, Staphylococcus aureus and Micrococcus luteus)and five Gram negative (Escherichia coli, Vibrio cholerae, Pseudomonas aeruginosa, Shigella dysenteriae and Shigella flexneri)bacteria by using different solvents namely petroleum ether, chloroform and ethanol. The result showed that all the solvent extracts exhibited considerable activity against the tested bacteria. The antibacterial activity increased with the increasing concentration of the extract. No antibacterial activity was noted at 10 mgml^{-1}, 20mgml^{-1}.

***KEY WORDS**: Gymnema sylvestre, antibacterial activity, leaf extract.*

INTRODUCTION

Plants have always played an important role for mankind especially as food and medicine. In the last few decades, there has been an exponential growth in the field of herbal medicine due to failure of modern medicine in providing effective treatment for chronic diseases and emergence of multidrug-resistant bacteria. Various extracts from traditional medicinal plants with folklore reputation have been examined [1-4] to identify the source of therapeutic drugs, but there is still an urgent need to screen novel substances that are bioactive towards pathogens with high resistance [5]

Gymnema sylvestre R.Br.(Asclepiadaceae) is a large,stout,much branched woody climber which grows predominantly in the tropical forest of central and south India and some parts of Africa[6]. It is used in the treatment of several diseases such as diabetes, corneal opacity, heart diseases, leucorrhoea, urinary infections, liver diseases, snake bite, stomach complaints and dental caries[7].Its roots are used as astringent, emetic, expectorant, refrigerant, stomachic and tonic [8,9]. In the present study, the selection of this plant for evaluation was based on its traditional usages.Although very few works have been done on the antimicrobial activity of this endangered medicinal plant[10,11],it needs further study for verification of its activity against disease-causing microorganisms. This paper describes the evaluation of the antibacterial potency of Gymnema sylvestre from West Bengal, India.

MATERIALS AND METHODS

Plant materials

The leaves of *Gymnema sylvestre* was collected in February, 2010 from Bolpur, West Bengal and authenticated by Prof.G.G.Maity, Department of Botany, University of Kalyani, Kalyani, West Bengal, India. The voucher specimen (SNS/Bot22) was deposited and preserved in the Department of Botany, University of Kalyani, Kalyani, and West Bengal, India.for reference.

Preparation of the plant extract

The leaves of *Gymnema sylvestre* were shed-dried at room temperature, powdered and passed through 60 mesh size sieves. Five hundred gram of powdered leaves were weighed accurately and extracted with various solvent selecting from nonpolar to polar, such as petroleum ether, chloroform and ethanol using soxhlet apparatus. To evaluate the antibacterial properties through agar well diffusion

method different extracts were taken in different concentrations(10 $mgml^{-1}$,20 $mgml^{-1}$, 50 $mgml^{-1,}$100 $mgml^{-1,}$ 200 $mgml^{-1}$) prepared in DMSO (Dimethyl sulphoxide).

Bacteria tested

The bacterial strains used to evaluate the antibacterial properties of different extracts of *Gymnema sylvestre* included *Bacillus subtilis, Staphylococcus aureus*, *Micrococcus luteus, Escherichi coli, Vibrio cholerae, Pseudomonas aeruginosa, Shigella dysenteriae* and *Shigella flexneri* . All the bacterial strains were obtained from IG and BG hospital, Kolkata. The bacteria were maintained on nutrient agar (Hi Media, India) slant at 4^0C and subcultured before use.

Antibacterial activity

The antibacterial test was performed using the agar well diffusion method [12].The test organisms were inoculated on nutrient agar plates and spread uniformly with the help of sterile glass spreader. On the nutrient agar wells of 5mm diameter were made using a sterile cork borer. The cut agar was carefully removed by the use of sterile forceps. To each well different concentration of plant extracts were added. Control experiment with DMSO was done on the same agar plate. The petriplates were incubated overnight at 37^0C . The antibacterial spectrum of the extract was determined in terms of zone sizes(inhibition zone diameters) around each well. Pure solvents were used as control for each bacterial strain. The experiment was repeated thrice and the average values were recorded for antibacterial activity.

Statistical Analysis

Simple correlation coefficient (r) values were calculated by the standard method [13].

RESULTS AND DISCUSSION

The results of antibacterial screening of crude petroleum ether, chloroform and ethanol extracts were shown in Tables1, 2 and 3 respectively. All the solvent extracts of *Gymnema sylvestre* leaf inhibited the growth of all the eight bacterial species tested in a dose-dependent manner. A significant positive correlation between the concentration of extract and inhibition zone diameter against bacteria supported the observation (Table4).The dose-dependent antimicrobial activity was also noted by other authors[14,15].However at 10mg/ml and 20mg/ml concentrations of the extract bacteria were found to be insensitive (Tables 1,2 and 3).

Table 1. Antibacterial activity of petroleum ether extract of leaf of *Gymnema sylvestre*

Concentration of Extract(mg/ml)	Inhibition zone diameter (mm)							
	Bs	Sa	Ml	Ec	Vc	Pa	Sd	Sf
10	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-
40	18	16	16	14	12	12	14	13
80	22	20	24	22	18	20	18	17
100	26	22	26	28	22	28	26	26
200	32	36	38	36	32	34	32	32

Bs *Bacillus subtilis*, Sa *Staphylococcus aureus*, Ml *Micrococcus luteus*, Ec *Escherichia coli*, Vc *Vibrio cholerae*, Pa *Pseudomonas aeruginosa*, Sd *Shigella dysenteriae*, Sf *Shigella flexneri*; - no inhibition zone

In the present investigation, the petroleum ether, chloroform and ethanol extracts exhibited nearly similar considerable antibacterial activity indicating the suitability of these solvents for dissolving most of the bioactive compounds of the plants. All the extracts highly affected the activity of Gram-positive bacteria in comparison to gram-negative bacteria. The susceptibility of Gram-positive bacteria towards various plant extracts than those of Gram-negative bacteria was also reported earlier [4,15]. The activity of *Gymnema sylvestre* leaf extract against both Gram-positive and gram-negative bacteria might indicate the presence of broad spectrum antimicrobial compounds. The present study support the traditional use of the plant in the treatment of several diseases. Further studies are required to identify and characterize chemical compounds present in leaf so that *Gymnema sylvestre* might be used as better alternative for synthetic antimicrobials.

Table 2. Antibacterial activity of chloroform extract of leaf of *Gymnema sylvestre*

Concentration of Extract(mg/ml)	Inhibition zone diameter (mm)							
	Bs	Sa	Ml	Ec	Vc	Pa	Sd	Sf
10	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-
40	18	18	20	14	13	14	12	14
80	22	24	24	24	20	22	18	18
100	36	32	28	28	24	30	26	22
200	38	38	36	32	30	32	34	30

Bs *Bacillus subtilis*, Sa *Staphylococcus aureus*, Ml *Micrococcus luteus*, Ec *Escherichia coli*, Vc *Vibrio cholerae*, Pa *Pseudomonas aeruginosa*, Sd *Shigella dysenteriae*, Sf *Shigella flexneri*; - no inhibition zone

Table 3. Antibacterial activity of ethanol extract of leaf of *Gymnema sylvestre*

Concentration of extracts($mgml^{-1}$)	Inhibition zone diameter(mm)							
	Bs	Sa	Ml	Ec	Vc	Pa	Sd	Sf
10	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-
40	16	17	20	12	13	14	12	13
80	18	20	24	20	18	22	22	18
100	32	32	36	26	20	30	28	24
200	36	38	39	34	32	38	32	30

Bs *Bacillus subtilis*, Sa *Staphylococcus aureus*, Ml *Micrococcus luteus*, Ec. *Escherichia coli*, Vc *Vibrio cholerae*, Pa *Pseudomonas aeruginosa*, Sd *Shigella dysenteriae*, Sf *Shigella flexneri*; - no inhibition zone.

Table.4 simple correlation coefficient (r) between different concentrations of extracts and Inhibition zone diameter exhibited by different bacteria.

Bacteria	Crude extract		
	Petroleum ether extract	Chloroform extract	Ethanol extract
Bs	0.9757	0.8387	0.8682
Sa	0.9964	0.9355	0.9115
Ml	0.9910	0.9892	0.8669
Ec	0.9573	0.8812	0.9573
Vc	0.9903	0.9504	0.9998
Pa	0.9257	0.8512	0.9483
Sd	0.9414	0.9564	0.8838
Sf	0.9335	0.9892	0.9540

Bs.*Bacillus subtilis*, Sa. *Staphylococcus aureus*, Ml *Micrococcus luteus*,Ec *Escherichia coli* , Vc. *Vibrio cholerae*, Pa.*Pseudomonas aeruginosa*, Sd. *Shigella dysenteriae and* Sf. *Shigella flexneri* . All values were statistically significant at 5% level.

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