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Full Length Research Paper

Screening of various extracts of *Gymnema sylvestre* (Retz.) R.Br. ex Schult. for antimicrobial activity

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Rising appearance of resistance to the presently existing antibiotics has necessitated sustained search for new antimicrobial compounds. The present study was designed to confirm ethno-medicinal assert of *Gymnema sylvestre* possessing antimicrobial activity that could be a superior alternative for synthetic antimicrobial agents, if proved to be successful enough. For this, the antimicrobial properties of *G. sylvestre* were tested against seven bacteria *(Shigella flexneri*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Enterobacter aerogenes)* and three fungi (*Aspergillus niger*, *Candida albicans*, and *Trichophyton rubrum)* by using different solvents like petroleum ether, chloroform, benzene, ethyl acetate, ethanol and distilled water. The result showed that all the solvent extracts exhibited considerable activity against the tested microorganisms. The antibacterial activity increased with the increasing concentration of the extract. Petroleum ether solvent extract of *G. sytvestre* is less active against all test microorganisms than five other solvent extracts, and distilled water extract is highly active against all test microorganisms. All solvent extracts at lower concentration of 50 mg/ 20 disc showed very smaller amount of activity against all test microorganisms.

Key words: *Gymnema sylvestre,* antibacterial activity, bacterial infections, medicinal plant.

INTRODUCTION

Plants have for eternity played a significant role for mankind mainly as food and medicine. In the last few decades, there has been an exponential increase in the field of herbal medicine for the treatment for chronic diseases. Various extracts from customary medicinal plants with folklore character have been examined (Awadh et al., 2001; Sinha and Biswas, 2010) to identify the basis of therapeutic drugs, but there is still a vital need to screen novel substances that are bioactive towards pathogens with high resistance (Cragg et al., 1997).

Gymnema sylvestre R.Br. (Asclepiadaceae) is a large, branched woody climber which grows mostly in the tropical forest of central and south India and some parts of Africa (Anonymous, 1997). It is known as Madhunashini, Gurmarbooti, and Meshashringi. 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 (Hiji Yasutake, 1990). Its roots are used as astringent, emetic, expectorant, refrigerant, stomachic and tonic (Uniyal, 1993; Selvanayagam et al., 1995). In the present study, the selection of this plant for estimate was based on its traditional usages. Although very small number of works have been done on the antimicrobial activity of this endangered medicinal plant (Satdive et al., 2003; Devi and Ramasubramaniaraja, 2010), it needs further study for verification of its activity against disease-causing microorganisms. This paper describes the evaluation of the antimcrobial potency of *G. sylvestre*

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from Rajasthan, India.

MATERIALS AND METHODS

Collection

Plant sample of *G. sylvestre* (whole plant) was collected from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan, in the month of July, 2009. These plants were used by these tribes in their daily lives to cure various ailments.

Identification

These samples were authenticated and submitted to the Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Sources of test organisms

Bacteria-pure culture of all test organisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri, Proteus vulgaris, Enterobactor aerogenes*) and fungi (*Candida albicans, Aspergillus niger*, and *Trichophyton rubrum)* were obtained, courtesy of Mahatma Gandhi Institute of applied Sciences (MGiaS), Jaipur, which were maintained on nutrient broth media.

Culture of test microbes

for the cultivation of bacteria, nutrient agar medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C for 25 to 30 min. Agar test plates were prepared pouring approximately 15 ml of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial\ cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24 to 48 h. To prepare the test plates, in bacteria, 10 to 15 ml of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of test extracts

Crushed powders of whole plant parts were successively Soxhlet extracted. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion; the extracts were cooled individually. Each filtrate was concentrated to dryness *in vitro* and re-dissolved in alcohol, until screened for antibacterial activity.

Bactericidal assay

For both, bactericidal *in vitro* disc diffusion method was adopted (Gould and Bowie, 1952) because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whattman No. 1 paper (6 mm in diameter), which contained three different concentration, its control (of the respective solvent) and tetracycline as reference drug (standard disc) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria, after which, the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

The inhibition zone (IZ) in each case were recorded and the activity index (AI) was calculated as compared with those of their respective standard reference drugs (AI = inhibition zone of test sample/inhibition zone of standard).

RESULTS AND DISCUSSION

The results of antimicrobial screening of crude petroleum ether, chloroform, benzene, ethyl acetate and ethanol and distilled water extracts are shown in Table 1. All the solvent extracts of *G*. *sylvestre* inhibited the growth of all the ten microorganisms species tested in a dose-dependent manner. The dose-dependent antimicrobial activity was also noted by other authors (Prescot et al., 2002; Vlietinck et al., 1995). Figure 1 shows the activity of all solvent extracts against all test microorganisms.

In the present investigation, the petroleum ether, chloroform, benzene, ethyl acetate and ethanol and distilled water extracts exhibited nearly similar considerable antimicrobial 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 bacteria and fungi. Extract of petroleum, ether, benzene and chloroform did not show activity against *S. aureus* bacteria. The antibacterial activity increased with the increasing concentration of the extract. Petroleum ether solvent of *G*. *sytvestre* is less active against all test microorganisms than five other solvent extracts, and distilled water extract is highly active against all test microorganisms. All solvent extracts concentration of 50 mg/20 disc showed a smaller amount of activity against all tested microorganisms.

The susceptibility of Gram-positive bacteria towards various plant extracts than those of Gram-negative bacteria was also reported earlier (Sinha and Biswas, 2010; Vlietinck et al., 1995). Some activities of *G*. *sylvestre* leaf extract study supports the traditional use of the plant in the treatment of several diseases (Sinha et al., 2010). Some intervention revealed that gurmar leaf powder had positive and encouraging effects over blood glucose levels (Paliwal et al., 2009). The activity of *G*. *sylvestre* extract against both bacteria and fungi might point out the occurrence of broad spectrum antimicrobial compounds. The present study ropes the traditional use of the plant in the treatment of numerous diseases. Advance studies are required to identify and characterize chemical compounds in attendance in this plant so that *G*. *sylvestre* might be used as a better alternative for synthetic antimicrobials.

Table 1. Antibacterial activity of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water extract of *Gymnema sylvestre*.

S/N	Solvents			*Sf*	*Sa*	*St*	*Pv*	*K p*	*P a*	*Ea*	*An*	*Ca*	*Tr*
1	Petroleum ether	A1	I.Z.	0	0	0	0	0	0	13	0	0	0
			A.I.	0	0	0	0	0	0	0.31	0	0	0
		A2	I.Z.	0	0	0	0	0	0	12	0	24.6	0
			A.I.	0	0	0	0	0	0	0.29	0	0.66	0
		A3	I.Z.	0	0	8	18	0	0	14.3	0	16	10
			A.I	0	0	0.26	0.40	0	0	0.34	0	0.43	0.35
		A4	I.Z.	0	0	10.3	19	0	0	18.3	0	22.3	10
			A.I.	0	0	0.34	0.43	0	0	0.44	0	0.60	0.35
2	Benzene	A1	I.Z.	0	0	8	7	7	8	10	0	8	10
			A.I.	0	0	0.26	0.15	0.17	0.50	0.24	0	0.21	0.35
		A2	I.Z.	0	0	10	9	9	7	16.6	0	7	7
			A.I.	0	0	0.33	0.20	0.22	0.43	0.40	0	0.18	0.25
		A3	I.Z.	0	0	9	8	8	6	12	7	8	8
			A.I.	0	0	0.30	0.18	0.20	0.37	0.29	0.29	0.21	0.28
3	Chloroform	A1	I.Z.	7	0	8	0	9	0	0	9	0	0
			A.I.	0.20	0	0.26	0	0.22	0	0	0.37	0	0
		A2	I.Z.	8	0	10	0	11	0	7	10.3	0	8
			A.I.	0.22	0	0.33	0	0.27	0	0.17	0.43	0	0.28
4	Ethyl acetate	A1	I.Z.	10	7	9	11	15	0	0	10.3	7	8
			A.I.	0.28	0.35	0.30	0.25	0.37	0	0	0.43	0.18	0.28
		A2	I.Z.	12	9	11	17	15	0	0	12	11	8
			A.I.	0.34	0.45	0.36	0.38	0.37	0	0	0.50	0.29	0.28
		A3	I.Z.	11	12	11	12	13	0	0	15	14	8
			A.I.	0.31	0.60	0.36	0.27	0.32	0	0	0.62	0.37	0.28
		A4	I.Z.	11	10	11	17	13	0	0	20	10	9
			A.I.	0.31	0.50	0.36	0.38	0.32	0	0	0.83	0.27	0.32
5	Methanol	A1	I.Z.	10.3	9.6	11	8.3	0	10.3	11	0	0	0
			A.I.	0.29	0.48	0.36	0.18	0	0.64	0.26	0	0	0
		A2	I.Z.	10.3	11.6	10	10	0	7	20	10.3	9	0
			A.I.	0.29	0.58	0.33	0.22	0	0.43	0.48	0.43	0.24	0
		A3	I.Z.	9.66	11	11	10	0	11	12	8.66	8.3	9
			A.I	0.27	0.55	0.36	0.22	0	0.68	0.29	0.36	0.22	0.32
		A4	I.Z.	11	11.3	11	13	0	0	10	9.3	8	10
			A.I.	0.31	0.56	0.36	0.29	0	0	0.24	0.38	0.21	0.35
		A5	I.Z.	9	11	11	10	0	0	12.3	11	8	9
			A.I.	0.25	0.55	0.36	0.22	0	0	0.30	0.45	0.21	0.32
6	Distilled water	A1	I.Z.	15	13	8	12	8	11	9	11	11	10
			A.I.	0.42	0.65	0.26	0.27	0.20	0.68	0.21	0.45	0.29	0.35
		A2	I.Z.	13	11	9	0	0	11	11	14	11	12
			A.I.	0.37	0.55	0.30	0	0	0.68	0.26	0.58	0.29	0.42
		A3	I.Z.	13	12	9	9	10.6	13	10	12	15	14
			A.I	0.37	0.60	0.30	0.20	0.26	0.81	0.24	0.50	0.40	0.50
		A4	I.Z.	12	13	10	8	0	11	11	14	13	12
			A.I.	0.34	0.65	0.33	0.18	0	0.68	0.26	0.58	0.35	0.42
		A5	I.Z.	15	13	7	10	8	14	13	15	12	15
			A.I.	0.42	0.65	0.23	0.20	0.20	0.87	0.31	0.62	0.32	0.53

I.Z. - Inhibition concentration of zone diameter (mm); A.I. - activity index, extract (mg/ml). Sf-*Shigella flexneri*, Sa- *Staphylococcus aureus*, St-*Salmonella typhi*, Pv- *Proteus vulgaris*, Kp- *Klebsiella pneumoniae*, Pa- *Pseudomonas aeruginosa*, Ee-*Enterobacter aerogenes*, An- *Aspergillus niger*, Ca-*Candida albicans*, Tr- *Trichophyton rubrum* ; 0 - no inhibition zone. A1- 50 mg/20 disc, A2- 100 mg/20 disc, A3-150mg/20 disc, A4- 200 mg/20 disc, A5-250 mg/20 disc.

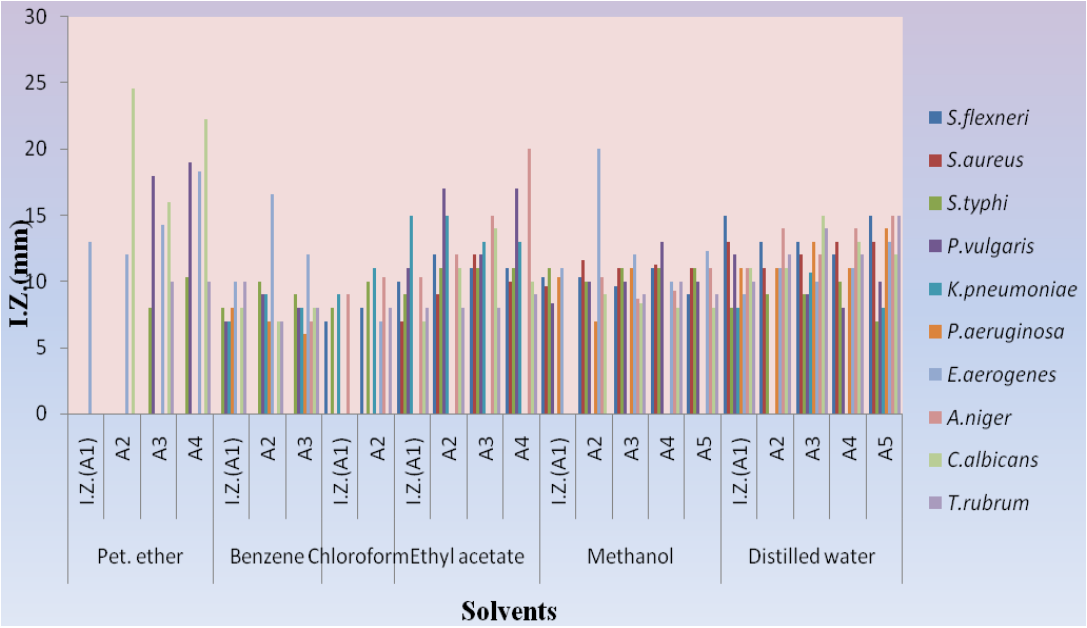

Figure 1. Antibacterial activity of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water extract of *Gymnema sylvestre*.

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