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Evaluation of antimicrobial activity of leaf extract of *Gymnema sylvestre*

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Abstract
The present study was designed with the main aim to evaluate antimicrobial activity of aqueous leaf extract of *Gymnema sylvestre* against pathogenic microorganisms. Leaves of *G. sylvestre* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with water. The extracts were diluted in dimethyl sulfoxide (DMSO) before testing the antimicrobial activity. The antimicrobial activity of extract for *B. subtilis, K. aerogenus, S. aureus, E. coli, A. niger and P. chrysogenium* was determined by agar well diffusion technique. The results of the present study revealed that aq. leaf extract of *G. Sylvestre* exhibited zones of inhibition at 100 mg/ml concentration. Aq. leaf extract of *G. sylvestre* exhibited zone of inhibition zone bacterial species like *B. subtilis* (10.55 mm), *K. aerogenus* (11.00 mm), *S. aureus* (8.50 mm), *E. coli* (12.50 mm), and against fungal species like *A. niger* (10.00 mm). In conclusion, present preliminary study delineated that the aqueous extract of *G. sylvestre* possesses considerable antimicrobial activities against *B. subtilis, S. aureus, E. coli, K. aerogenes and A. niger*. Hence, further experiments like isolation and purification of phytoactives of *G. sylvestre* responsible for antimicrobial activities could be recommended to carry out to further enhance the antimicrobial activities of phytoactives present in aq. leaf extract of *G. sylvestre*.

Keywords: *Gymnema sylvestre*, water extract, leaves, antimicrobial activity, zone of inhibition, pathogenic microorganisms

Introduction
As per World Health Organization (WHO) therapeutic plants would be the best source to acquire an assortment of medications [1]. The data on therapeutic plants from Ayurveda, Unani, Homeopathy and Siddha gives thought that the medicinal plants contain a wide range of ingredients. The medicinal value of plants is because of ingredients like alkaloids, flavonoids, tannins and phenolics [2]. They can be used to treat chronic as well as infectious diseases. Medicinal plants are the main source of pharmaceuticals and healthcare products [3]. Medicinal plants products are used as home remedies to treat specific conditions as well as complex preparations to treat life threatening diseases [4]. The antimicrobial properties of medicinal plants have been examined by some of scientists around the world. Recent exploration survey uncovered that, medicinal plants are evaluated for biological activities for discovering likely new compounds for remedial use [5-7]. The utilization of plant extracts and phytochemicals, both with known antimicrobial properties, can be of incredible importance in therapeutic medicines [8]. Thus, more investigations relating to the utilization of plants as therapeutic agents thought to be underscored.
G. sylvestre (Asclepiadaceace), a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India. The plant is considered to be a good source of a large number of bioactive substances. G. sylvestre leaves contains large number of phytochemicals like terpenoids, saponins, gymnemic acids, gymnemasaponins. The essential oil obtained from G. sylvestre leaves exhibited antioxidant and antimicrobial activity [9]. There is a growing demand for G. sylvestre leaves in pharmaceutical trade. The active compound gymnemic acid was extracted from leaves and used widely as an antidiabetic, anti-sweetner and anti-hypercholesterolemia.

It also has stomachic, diuretic and cough suppressant properties [10, 11]. Thus, literature study evidenced the traditional usage of G. sylvestre in pharmaceutical industry. However, very limited work has been done on the antimicrobial activity of this medicinal plant. Hence, the present study was designed with the main aim to evaluate antimicrobial activity of aqueous leaf extract of G. sylvestre against pathogenic microorganisms.

Materials and Methods

Plant material

The leaves of G. sylvestre were collected from natural habitat at Chikkaballapura District, Karnataka, India. The plant was identified by Mrs. Nalini Thimmappa Jayaramappa, Assistant Professor, Dept. of Botany, Maharani Cluster University, Bengaluru, Karnataka, India.

Preparation of plant extract

Leaves of G. sylvestre was washed thoroughly under running tap water, dried on paper. Dried leaves were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with water. Extract was concentrated by distilling the solvent in a rotary flash evaporator. The extract was preserved in airtight containers and stored at 4-5 ^{o}C until further use. The extract was diluted in dimethyl sulfoxide (DMSO) before testing for the antibacterial activity [12].

Procurement of cultures

For Antimicrobial activity studies following microbial cultural were used. *Bacillus subtilis* (ATCC 2239), *Escherichia coli* (ATCC 25744), *Staphylococcus aureus* (ATCC 2178), *Klebsiella aerogenes* (ATCC 2239), *Aspergillus niger* (ATCC 504) and *Penicillium chrysogenum* (ATCC 709). The microbial cultures were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune.

Antimicrobial activity

Antimicrobial activity was carried out by agar well diffusion method.13 Pure cultures of Bacillus subtilis, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes* and the fungal cultures of *Aspergillus niger* and *Penicillium chrysogenum* were obtained National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune. The mother cultures of Bacillus subtilis, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Aspergillus niger* and *Penicillium chrysogenum* were allowed to stand for 24 h in order to reach the stationary phase of growth before the assays. Petri dishes containing the mother cultures with proper sterile Muller-Hinton agar medium was used for bacteria. The media were inoculated to obtain the microorganism concentration of 130 x 107 cfu/ml. A sterile filter paper disc was loaded with 40 ml sample (50 mg/ml). The disc was placed near the edge of the agar surface of the inoculated plate. All the plates were kept at 50 °C for half an hour for diffusion. The plates were then incubated for 24 h at 37 °C and the diameters of growth inhibition zones were measured using distilled water as a blank. Each assay was performed in triplicates on three independent experimental runs. The minimum inhibitory concentration (MIC) of extracts indicating clear inhibition was determined by agar diffusion method [14]. Chloramphenicol (10 µg/ml) was used as standard for the antimicrobial activity.

Results and Discussion

As compared with synthetic drugs, naturally derived drugs are therapeutically active and commercially available [15]. The antimicrobial activity and MIC ranges between 10.00 mg/ml to 100 mg/ml exhibited by aq. leaf extract of G. sylvestre extract was as represented in Table 1 and Table 2 respectively. In the antimicrobial studies aq. leaf extract of G. Sylvestre exhibited zones of inhibition at 100 mg/ml concentration. Aq. leaf extract of G. sylvestre exhibited zone of inhibition zone bacterial species like Bacillus subtilis (10.55 mm), K. aerogenus (11.00 mm), S. aureus (8.50 mm), E. coli (12.50 mm) and against fungal species like A. niger (10.00 mm). However, aq. leaf extract of G. sylvestre did not exhibited any zone of inhibition against fungal species P. chrysogenium. These findings delineated that aq. leaf extract of G. sylvestre was active against tested organisms. Hence, the present results of the present research investigation offer a scientific basis for traditional use of aq. leaf extract of G. sylvestre.

Table 1: Antimicrobial activity of aq. leaf extract of *G. sylvestre* against various microbial strains

Microorganisms		Aq. leaf extract of *G. sylvestre*
Gram Positive Bacteria	*Bacillus subtilis*	10.50
	Klebsiella aerogenus	11.00
Gram Negative Bacteria	*Staphylococcus aureus*	8.50
	Escherichia coli	12.50
Fungal Species	*Aspergillus niger*	10.00
	Penicillium chrysogenium	-

Table 2: MIC of aq. leaf extract of *G. sylvestre* against various microbial strains

Microorganisms		Aq. leaf extract of *G. sylvestre*
Gram Positive Bacteria	*Bacillus subtilis*	11.50
	Klebsiella aerogenus	11.50
Gram Negative Bacteria	*Staphylococcus aureus*	11.50
	Escherichia coli	12.50
Fungal Species	*Aspergillus niger*	10.50
	Penicillium chrysogenium	-

Plants are sources of very potent and powerful drugs with antibacterial properties [16, 17]. Antibacterial assay of Zulu medicinal plants showed that methanolic extracts of Chelianthes viridis, Dioscorea dregeanam, Dioscoria silvatica and Molianthus cosnlosus exhibited activity against both Gram positive and Gram-negative bacteria [18]. Samy and Ignacimuthu screened 30 Indian folk medicinal plants used by traditional healers using disc diffusion method. Among them, the leaf extracts of *Cassia occidentalis* and *Cassia comiculata* exhibited significant broad-spectrum antibacterial activity against *Bacilus subtilis* and *Staphylococcus aureus* [19]. Rupanar *et al*. literature report evidenced the antioxidant and antimicrobial activity of G. sylvestre leaf and stem [20]. Our findings are comparable to literature findings reported by various research investigators. In the present study, antimicrobial activity of aq. leaf extract of G. sylvestre was evident due to clear zone of inhibition against test organisms like B. subtilis, S. aureus, E. coli, K. aerogenes, A. niger. The antimicrobial activites of aq. leaf extract of G. sylvestre could be mainly accredited to phytoactives of phytochemicals present in different proportions in G. sylvestre.

Conclusion

The present preliminary study delineated that the aqueous extract of G. sylvestre possesses considerable antimicrobial activities against B. subtilis, S. aureus, E. coli, K. aerogenes and A. niger. Hence, further experiments like isolation and purification of phytoactives of G. sylvestre responsible for antimicrobial activities could be recommended to carry out to further enhance the antimicrobial activites of phytoactives present in aq. leaf extract of G. sylvestre.

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