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Assay of Antibacterial Activity of *Gymnema sylvestre* Extracts

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ABSTRACT
The aim of the present study was to determination of antibacterial activities of ethanolic and aqueous leaf extracts of Madhunaashini *(Gymnema sylvestre).* Leaves of *G. sylvestre* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with water and ethanol. The extracts were dissolved in dimethyl sulfoxide (DMSO) before testing the antibacterial activity. The antibacterial activity of leaf extracts *G. sylvestre* for *Proteus mirabilis, E. coli and Pseudomonas aeruginosa* was determined by agar well diffusion technique. Results delineated that zone of inhibition of *G. Sylvestre* for ethanolic & aqueous extracts for *E. coli* and *P. mirabilis* was found to be 17 mm & 13 mm and 19 mm & 16 mm respectively. Leaf extracts *G. sylvestre* for *P. aeruginosa* exhibited the zone of inhibition as 10 mm for ethanolic extract. In addition, ethanolic extract of *G. Sylvestre* exhibited antibacterial activity (19 mm) against *P. mirabilis* which is at par with that of standard antibiotic Streptomycin (20.5 mm). In conclusion, *G. sylvestre* could be used as an antibacterial agent since it was demonstrated from the current study findings that the ethanolic extract of *G. sylvestre* exhibited antibacterial activity at par with that of standard antibiotic streptomycin.
Keywords: *Gymnema sylvestre*, Antibacterial agent, *P. mirabilis*

1. Introduction
Indian flora accounts for about 45,000 plant species out of which several thousands have pharmacological significance [1]. Plants are a great concern for drug discovery exploration and a major source of our modern medicine. About 25% of modern medicines are derived from a plant source and merely 5-15% of plants have been investigated for their medicinal use [2]. Nowadays, natural plants, herbal medicines, phytomedicines and functional foods are extensively studied by scientists all over the world which resulted with the lucrative therapeutic potentials such as antidiabetic [3-6], antiobesity and lipid lowering [6], anti-inflammatory [7], and anti-bacterial activities [8].
Among the potential medicinal plants, *Gymnema sylvestre*, belongs to the family of Apocynaceae and is traditionally used for the treatment of various diseases. The leaves of gurmar are of tremendous medicinal importance due to its unique property to directly mask the tongue's ability to taste sweet foods; at the same time suppresses glucose absorption from the intestine. This is the reason it is known in Hindu word as "gurmar" or "destroyer of sugar" [1]. Aroa and Kaur assayed the antibacterial activity of certain spices and revealed that spices have a great potential to be used as antibacterial agents [9]. Bagchi et al reported that seeds of *Coprophilous* plants are effective in subsiding the pathogenic organisms of animals as well as humans; Furthermore, with water solubility and non-poisonous nature of seeds of *Coprophilous* plants are proven to be a potential source of antimicrobial drugs [10]. On screening eight Nigerian medicinal plants used traditionally in the treatment of infectious diseases in both humans and animals as antibacterial activity, *Angeiossus schimperi* and *Anacardium occidentale* exhibited significant activity against *Escherichia coli* and *Pseudomonas aeruginosa* [11]. The results of antibacterial screening of six Moroccan medicinal plants showed that n-butanol extract of *Calotropis procera* was most effective

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against the eight pathogenic bacteria tested [12]. Plants have been used for the treatment of various diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs [13]. Thus over 50% of these modern drugs are of natural products of origin and as such play an important role in drug development in the pharmaceutical industry [14]. Infectious diseases are the number one cause of death world-wide and in tropical countries it accounts for approximately 50% of deaths. This may be due to poverty and increasing incidence of multiple drug resistance organisms. *G. sylvestre* (Asclepiadaceae) is a large tropical liana native to central and western India. There is a growing demand for *G. sylvestre* leaves in pharmaceutical trade. The active compond gymnemic acid was extracted from leaves and used widely as an anti-diabetic, anti-sweetner and anti-hypercholesterolemia. It also has stomachic, diuretic and cough suppressant properties [15]. Literature study evidenced the traditional usage of *G. sylvestre* in pharmaceutical industry. However, reports on antibacterial activity of *G. sylvestre* are scarce. With this background current study was aimed to evaluate the antibacterial activity of *G. sylvestre* against pathogenic microorganism.

2. Materials and Methods

2.1 Plant material

The leaves of *G. sylvestre* were collected from local provinces at Chikkaballapura District of Karnataka State.

2.2 Extract preparation

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). The extraction was done with different solvents in their increasing order of polarity such as water and ethanol. Each time the material was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at 4-5^{o} C until further use. The extracts were dissolved in dimethyl sulfoxide (DMSO) before testing for the antibacterial activity [16].

2.3 Pathogenic microorganisms

The multiple antibiotic-resistant pathogenic microorganisms viz. *Escherichia coli, Proteus mirabilis,* and *Pseudomonas aeruginosa* were isolated from clinical samples of local hospital in and around Chikkaballapura district headquarter and confirmed by various microscopic evaluation like Gram's staining [17]. Motility, capsule and spore formation as per the procedure prescribed by Collins and Lyne [18]. All the bacterial pathogens were further confirmed by suitable biochemical tests and used for antimicrobial activity studies [19].

2.4 Antibacterial activity

The antibacterial activity of leaf extracts of *G. sylvestre* was determined by agar well diffusion technique. Muller Hintor agar plates were spread with an overnight culture of each bacterial strain. The well was made by sterile standard cork borer and 100 mg/ml solution of extract added to each well. Then bacterial plates incubated at 37^{0}C for 24 hours after which diameter of zones of inhibition were measured (mm) by using Hi Antibiotic Zone Scale-C (Himedia). Each assay was performed in triplicate and means values are reported. Standard antibiotic strip of Streptomycin (100 µg/disc) for each bacterium along with DMSO were used as positive and negative controls respectively.

3. Results and Discussion

Plants have played a pivotal role for mankind mainly as food and medicine. Medicinal plants have been used for many centuries for human diseases because they contain bioactive components of therapeutic value because of their antimicrobial properties and they contain secondary metabolites such as alkaloids,

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phenolic compounds, etc...[20]. Countries like India have been using crude plants as medicine since Vedic period.

The dried leaf powder of *G. sylvestre* was subjected to successive solvent extraction in their increasing order of polarity such as hot water and ethanol. The extracts were concentrated and dissolved in DMSO for determination of the antibacterial activity [16]. Pathogenic microorganisms like *P. mirabilis, E. coli* and *P. aeruginosa* and their zone of inhibition was compared with stander antibiotic streptomycin. The zone of inhibition of *G. Sylvestre* for ethanolic and aqueous extracts was for *P. mirabilis* & *E. coli* was found to 17 mm & 14 mm and 17 mm & 14 mm respectively. *P. aeruginosa* exhibited the zone of inhibition 8 mm for ethanolic extract. Among the leaf extracts of *G. Sylvestre*, ethanolic extracts showed excellent antibacterial activity than aqueous extracts. Further it was observed that ethanolic extract of *G. Sylvestre* possess antibacterial activity (17 mm) against *P. mirabilis* which is equal to that of standard antibiotic Streptomycin (18.5 mm). The results of antibacterial activity of *G. Sylvestre* was represented in Table 1.

Table 1: Antibacterial activity of leaf extracts of *G. sylvestre*

S. No.	Bacterial strains	Leaf extracts of *G. sylvestre*		
		Ethanolic extract	Aq. extract	Streptomycin
		Zone of Inhibition (mm)		
1.	*E. coli*	17.00	13.00	25.00
2.	*P. mirabilis*	19.00	16.00	20.50
3.	*P. aeruginosa*	10.00	-	23.00

Plants are sources of very potent and powerful drugs with antibacterial properties [21,22]. 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 [23]. 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* [24]. In the present study, antibacterial activity of solvent extracts of leaves of *G. sylvestre* was evident due to clear zone of inhibition against test organisms like *E. Coli, S. aureus and Klebsiella Sp.* the antibacterial activity of *G. Sylvestre* was due to different class of phytochemicals in different proportions.

Literature reports evidenced antibacterial activities of *G. sylvestre.* Saumendru reported that *G. sylvestre* leaf extracts showed good prospects as an antibiotic herbal remedy since it was effective as herbal formulation for the treatment of microbe's related infections [25]. Bhuvaneswari et al reported that the methanol extracts in acidic range have good activity towards all the pathogens showing its broad-spectrum nature [26]. Furthermore, Satdive et al reported the antimicrobial effect of ethanolic extract of *G. sylvestre* against *B. pumilus, B. subtilis, P. aeruginosa* and *S. aureus* [27]. In summary, methanolic and ethanolic leaf extract of *G. sylvestre* possesses considerable antibiotic and antimicrobial activity.

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CONCLUSION

The study findings confirmed that the ethanolic extract of Madhunaashini (*Gymnema sylvestre)* exhibited antibacterial activity at par with that of standard antibiotic streptomycin. Therefore, current study supplies as a scientific evidence-based report for the traditional use of *Gymnema sylvestre* as an antibacterial agent.

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