

Qualitative phytochemical analysis and antimicrobial activity studies of *Gymnema sylvestre* R. Br.

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ABSTRACT

Gymnema sylvestre R. Br., a medicinal plant belonging to the family Asclepiadaceae, is an important antidiabetic medicinal plant, commonly known as gudmar. The present study was undertaken to analyze the phytochemical components and for screening the antimicrobial activity of leaf extracts of *Gymnema sylvestre*. The qualitative analysis indicated the presence of alkaloids, phenolic compounds, flavonoids, saponins, cardiac glycosides, whereas sterols and anthraquinone glycosides were found to be absent in the methanolic extracts. Antimicrobial activity of *G. sylvestre* leaf extracts was studied. Aqueous and methanolic leaf extracts were studied against *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus* and *Candida albicans* for their antimicrobial efficacy. Tetracycline (0.1mg/ml) was used as positive control and methanol was used as negative control. The methanolic extract of the leaves were showing activity against all four tested microorganisms while aqueous leaf extracts found to be non effective. The antimicrobial activity was present for all four tested microorganism. The result of present investigation clearly indicates the antibacterial and antifungal activity of the plant and ascertains the value of this plant which could be of considerable interest to the development of new drugs.

Keywords: *Gymnema sylvestre*, antidiabetic, antimicrobial activity, phytochemical analysis, aqueous and methanolic extracts.

Gymnema sylvestre R. Br. is a medicinal plant belonging to the family Asclepiadaceae. *G. sylvestre* is regarded as one of the plants with potent antidiabetic properties. It has been used as a natural treatment for diabetes for nearly two millennia and commonly known as gudmar. The plant has been reported to possess antimicrobial [1], antieurodonic [2] and antiviral effects. It is also used for controlling obesity in the form of *Gymnema* tea. The active compound of the plant is a group of acids termed as gymnemic acid. Secondary metabolites like alkaloids, terpenoids, phenolics, steroids and flavonoids play an important role in interaction of the plant with its environment [3]. The amount and type of phytochemical compounds vary from plant to plant. In *Gymnema* species a number of phytochemical constituents have been reported. Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects [4,5]. Saponins possess hypocholesterolemic and antidiabetic properties [6]. The terpenoids have also been shown to decrease blood sugar level in animal studies. The steroids and saponins are responsible for central nervous system activities [7]. More recently, drug discovery techniques have been applied to the standardization of herbal medicines to elucidate analytical marker compounds [8].

Antimicrobial efficacies of different *Gymnema* species have been reported. Diarrheagenic serotypes of *Escherichia coli* cause serious food poisoning in humans. *Serratia marcescens* is a motile, short rod-shaped, facultative anaerobe bacterium, classified as an opportunistic human pathogen. *Staphylococcus aureus* is a facultative anaerobic human pathogenic bacterium. Infections due to *Staphylococcus aureus* have continued to be a major source of morbidity and mortality in hospitals and these organisms are now exhibiting multi-drug resistance to commonly used antibiotics, hence a significant cause of concern among physicians [9]. *Candida albicans* is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans [10]. Antibacterial potentials of the aqueous and organic solvents extracts from powdered leaves of *Gymnema kollimalayanum* were reported against Gram-negative and Gram-positive bacterial strains [11]. The present study was aimed at the qualitative analysis of the phytochemical composition and for the antimicrobial screening of crude leaf extracts of *G. sylvestre* against some Gram-positive and Gram-negative bacterial strains and fungus.

The plants of *G. sylvestre* R.Br. (Asclepiadaceae) were collected from commercial nursery in Pune, India. Leaves were obtained from well grown healthy plants. Shade dried leaves were powdered using mixer grinder. Filtered leaf extracts were concentrated and were subjected to qualitative tests for the identification of various phytochemical constituents like alkaloids, flavonoids, saponins, cardiac glycosides, sterols, steroids and anthraquinone glycosides as per the standard protocol [12, 13]. Methanolic and aqueous extracts of the leaves were subjected to phytochemical analysis. Separation of the secondary metabolites such as alkaloids, flavonoids, saponins, cardiac glycosides and sterols from the leaves of *G. sylvestre* was carried out by thin layer chromatography [14]. For antimicrobial studies extraction of plant material was prepared by cold percolation method. The air-dried and powdered plant material, 5 g of each, was soaked in 50 ml methanol and kept for 48 hrs with intermittent shaking. Filtered and dried plant extracts were dissolved in 1 ml dimethyl sulfoxide (DMSO) and 50µl and 100µl of each sample was taken for experiment. The methanolic and aqueous extract of plant materials were screened against *Escherichia coli* (Gram-negative), *Serratia marcescens* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Candida albicans* (Fungus). The *in vitro* antibacterial and antifungal activity of the leaf extracts was evaluated by modified disk diffusion method using NA and SDA medium plates. Filter paper discs of 6 mm diameter were prepared and sterilized. The discs were impregnated with 50 µL and 100 µL of the plant extracts and were placed on the inoculated agar. Plates were kept for incubation at 37°C for 24 h for bacterial strains and 48 hrs for fungus. Tetracycline (0.1mg/ml) was used as the positive control and methanol as the negative control. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated two times.

Investigations on the phytochemical screening of *G. slyvestre* leaf extracts revealed the presence of alkaloids, flavonoids, cardiac-glycosides, steroids and saponins whereas the metabolites, anthraquinones and sterols were found to be absent in the methanolic extracts. Similar results are reported in *Gymnema* by Kalidas and Mohan [15]. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. The main active compound of *G. sylvestre* is gymnemic acid, saponins and oleanane type of triterpenoid [16]. These phytochemicals may be responsible for different therapeutic properties of *Gymnema* like anti-diabetic, anti-oxidant, anti-pyretic and antimicrobial role. Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [17]. Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities [18]. The terpenoids have also been shown to decrease blood sugar level in animal studies. Steroids and triterpenoids showed the analgesic properties. Saponin is used as mild detergents and in intracellular histochemical staining. It is also used to allow antibody access in intracellular

proteins. In medicine, it is used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory, weight loss, etc. Saponins are known to have antifungal properties. TLC technique is reported to be effective in generating metabolite profiles of various plant compounds and their purification. In the present studies thin layer chromatographic screening of the metabolites, indicated the separation at different Rf values. The alkaloids separated showed two fractions (Rf =0.84 and 0.84) and saponins three (Rf= 0.89, 0.84, 0.81) under visible light. TLC chromatogram for cardiac glycosides showed the separation of four fractions (Rf =0.90, 0.69, 0.58, 0.31) and two bands of flavonoids (Rf =0.80 and 0.84). The sample concentration 10 µl is found to be showing more bands in case of saponins and cardiac glycosides whereas 5 µl of sample concentration was sufficient to generate the bands in case of alkaloids and flavonoids. Sukesh et al. reported the presence of steroids/terpenoids and coumarins by using thin layer chromatography of hexane and chloroform extract of *G. sylvestre* [19]. The presence of different active fractions in the TLC studies further reiterates the antibacterial properties.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antimicrobial activity assay. The results of the present studies (Table 1) indicated that the plant *Gymnema* had great potential of antimicrobial activities against the Gram-positive and Gram-negative bacteria and the fungal species tested. The methanol extracts indicated a zone of inhibition comparable to that of the standard antibiotic, tetracycline used, whereas the aqueous extracts were found to be non effective. This is in agreement with the previous reports which show that aqueous extracts of plant generally showed little or no antibacterial effects [20, 21]. Generally the methanol extract had the highest activity against both bacterial and fungal isolates [22]. The result revealed a lower activity of the extracts against *Serratia marcescens* and was effective at a concentration of 100 µL whereas for *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, zone of inhibition was comparable by using both 50 µL and 100 µL of the extract. The relatively high antimicrobial activities of the plant extracts are most likely due to the presence of high concentrations of the secondary compounds with antimicrobial properties. Antimicrobial activity of an ethanolic extract of *G. sylvestre* leaves are demonstrated against *Bacillus pumilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [1]. Quinlan et al. [23] worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Naturally occurring flavonoids and polyphenolic compounds have antimicrobial and resistance modulating potentials [24].

Table 1. Antimicrobial activity of *Gymnema sylvestre* leaf extracts on pathogenic microorganisms.

Concentration of leaf extracts	Zone of inhibition (cm)			
	<i>Escherichia coli</i>	<i>Serratia marcescens</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Positive control	1.7	0.9	2.1	2.1
Negative control	0.0	0.0	0.0	0.0
100µL (methanol)	1.4	0.7	1.8	1.8
100µL (Water)	-	-	-	-
50µL (methanol)	1.3	0.1	1.6	1.4
50µL (Water)	-	-	-	-

Large scale isolation of secondary metabolites from *G. sylvestre* can be predicted to remain an essential component in the search for new secondary metabolites and its pharmacological activities. *G. sylvestre* leaf extracts exhibit broad spectra of antimicrobial activity. The result of present investigation clearly indicates the antibacterial and antifungal activity of *G. sylvestre* leaves and

ascertains the value of this plant used in ayurveda, which could be of considerable interest to the development of new drugs. The result of this study supports the use of plants as therapeutic agents for the treatment of several diseases caused by the pathogenic bacterial and fungal populations.

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