

Original Research Article

# Phytochemical analysis and antibacterial activity of *Gymnema sylvestre* leaf extracts

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## Abstract

Over the past ten years, the use of natural medicine has expanded and popularized worldwide. Herbal medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system. 80% of the world's population depends on the herbal medicines to serve their. *Gymnema sylvestre* is an herb native to the tropical forests of southern and central India and Sri Lanka. It belongs the family *Asclepiadaceae*. Chewing the leaves suppresses the sensation of sweet. This effect is attributed to the presence of the compound named as gymnemic acids. The results of present study clearly indicate the presence of different phytochemical compounds and antibacterial activity of the aqueous and methanolic extracts of leaves. 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 the plant activities. Presence of these compounds of plant which could be of considerable interest to the development of new drugs.

**Keywords:** *Gymnema sylvestre*, Asclepiadaceae, Gymnemic acids, Drugs.

## Introduction

Natural medicine has been used for centuries as remedies for human diseases because they contain valuable therapeutic components [1]. For the past few years, secondary metabolites (phytochemicals) have been investigated extensively as a source of medicinal agents. It is evaluated that phytochemicals with good antibacterial activity will be used for the treatment of microbial infections [2]. These phytochemical compounds have made a great contribution in maintaining human health. The importance of drugs produced from plants cannot be expressed with the recent trend of high percentage of resistance of microorganisms to the present day antibiotics [3]. *Gymnema sylvestre* belongs to the family asclepiadaceae. It is a very important medicinal plant with branched woody climber, distributed throughout India. It is popularly known as 'Gudmar' (gud-jaggary, mar-kills) for its distinctive property of temporarily destroying the taste of sweetness. Leaves are tasteless with a faint pleasant aromatic

smell and used as a remedy for diabetes and other health problems such as stimulant, laxative, stomachic, diuretic, biliousness, sore eyes and cough. Studies on its pharmacological properties have shown that *G. sylvestre* posses antihyperglycemic effect, anti atherosclerotic, hepatoprotective activity, larvicidal effect and antimicrobial effects [4]. Our present study investigated phytochemical compounds and antimicrobial activity of aqueous and methanolic extracts of *G. sylvestre* leaves.

## Materials and Methods

### Collection and authentication of plants

The leaf material of *Gymnema sylvestre* was collected from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad, Andhra Pradesh state. The plant was authenticated at the Department of Botany, Osmania University, Hyderabad.





Figure. 1. *Gymnema sylvestre*

### Preparation of plant material

The collected leaf material was washed thoroughly under running tap water and air dried under shade at room temperature for a period of eight days. The dried plant was then homogenized into powder using the laboratory mortar and pestle.

### Process of plant extraction

50g of the leaf powder was placed in conical flask and soaked in 500ml of the aqueous and methanol separately covered with cotton wrapped with aluminium foil. The flasks were allowed to stay and shaken daily for 5 days after which they were filtered using muslin cloth. The filtrate was evaporated until it was paste like using the laboratory steam bath. A sticky dark semi-solid extracts were obtained. It was weighed and stored in sterile container and kept in the refrigerator at 4° C for future use [5].

### Primary screening of phytochemicals

The preliminary phytochemical screening of the leaf extracts were conducted in the Department of Genetics, Osmania University, Hyderabad. The plant extracts of both solvents were analyzed for the presence of alkaloid, flavonoids, steroid, terpenes, tannins, saponins, glycosides, anthraquinone in line with the standard procedures as described [3, 4, 6-10].

### Preliminary Phytochemical Screening

The phytochemical screening of the extracts was done using standard procedure as described [8]. The following qualitative tests were carried out as follows.

#### Steroids and Terpenoids

8mg of the extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of conc Sulphuric acid. Blue colour in chloroform layer which changes to green shows the presence of steroids, whereas the appearance of pink colour in chloroform layer shows the presence of terpenoids.

#### Flavonoids

8mg of the extract was dissolved in methanol. Magnesium turnings were added into this followed by conc HCL. A magenta colour shows the presence of Flavonoids.

#### Coumarins

8mg of the extract is dissolved in methanol and alcoholic KOH was added. The appearance of yellow colour which decolorizes while adding conc HCL shows the presence of Coumarin.

#### Phenolic compounds

Plant extract was dissolved in alcohol and a single drop of neutral ferric chloride was added to this. The intense colour indicates the presence of phenolic compound.

#### Tannins



8 mg of the extract was boiled with 1 ml water for 30 min. The extract is filtered clear and to this 0.5 ml 2% gelatin was added. A curdy white precipitate indicates the presence of tannin.

### Alkaloids

8mg of the extract was dissolved in conc. HCL and filtered. A few drops of solution are poured into the center of watch glass. Mayer reagent is added along the sides of the watch glass with the help of a glass rod. Formation of a gelatinous white precipitate at the junction of two liquid shows the presence of alkaloids.

### Saponins

Plant extract was dissolved in water and shaken well. Long time presence of froth shows the presence of saponins.

### Terpenoids

8mg of the extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of conc Sulphuric acid. The appearance of pink colour in chloroform layer shows the presence of terpenoids.

### Preparation of extracts

The crud extracts of leaf material was reconstituted by using the DMSO and distilled water for the antimicrobial assay to obtain 60mg/ml, 40mg/ml, and 20mg/ml concentrations. These were obtained by dissolving 0.3g, 0.2g and 0.1g of the extract in 5ml (1:1) of the DMSO and distilled water. The reconstituted extracts were then stored at 4°C in sample bottles until required.

### Test organisms

The standard bacterial culture (Gram-negative *Escherichia coli* (E. coli), *Klebsiella pneumonia* (*Klebsiella*) and Gram-positive *Bacillus subtilis*) was collected on nutrient agar slants from the Department of Microbiology, Osmania University, Hyderabad, Andhra Pradesh.

### Antimicrobial activity by disc diffusion method

LB agar (16 g/l agr) was dissolved in distilled water and made up to mark in a litre standard flask. The mixture LB agar was sterilized in an autoclave for 15 mins at 121°C and allowed to cool to 47°C in. The prepared agar was then poured into Petri dish and allowed to cool to room temperature for solidify in the laminar airflow. The discs were prepared from Whatman filter paper (No. 1) using the office perforator and placed on the solidified LB agar plates. The method was described and used Bauer et al., (1996) [7]. Standard 6mm diameter dish were prepared. From the stock solution of aqueous and methanol extracts, 60mg/ml, 40mg/ml, and 20mg/ml concentrations of 0.2ml plant extract at the tested concentrations was added to 20 discs each separately. The same

concentration of standard antibiotic tetracycline aqueous solution was added as positive control to the disc. Allowed them to dry in the laminar air flow. The negative control was DMSO. Maximum of absorption capacity of each disc was expected to have 0.01cm<sup>3</sup> [5]. The set up was then placed in an incubator for 24h in the inverted position. The bottom of each petridis was marked with marker.

The experiment was in triplicates at each dosage. After about 24h of incubation, they were removed from the incubator and examined for growth. Zone of inhibition in mm was measured with a transparent ruler. Zone of inhibition was measured by comparing with the standard controls. Activity was scored by recording the lowest concentration which prevented growth, called minimum inhibition concentration (MIC).

## Results and Discussion

### Preliminary phytochemical screening of *Gymnema sylvestre* leaf extracts

The constituents of phytochemicals compounds were observed in the aqueous and methanolic extracts of *Gymnema sylvestre*. Except Iridoids all compounds such as Steroids, Flavonoids, Iridoids, Coumarins, Phenols, Tannins, Alkaloids, Saponins, Terpenoids were present in the methanolic extracts of *Gymnema*. Only saponins were observed in aqueous extracts of *Gymnema* (Table 1). The presence of all these compounds in the plants, they are responsible for different medicinal properties such as antioxidant, antimicrobial, antiinflammatory and anticancer activities etc. Earlier studies have revealed that the most important bioactive compounds of medicinal plants are alkaloids, flavonoids, tannins, and phenolic compounds [12].

S.No	Phytochemical compound	Aqueous extracts	Methanolic extracts
1.	Steroids	-	+
2.	Flavonoids	-	+
3.	Coumarins	-	+
4.	Phenols	-	+
5.	Tannins	-	+
6.	Alkaloids	-	+
7.	Saponins	+	+
8.	Terpenoids	-	+

Table. 1. Phytochemical screening of aqueous and methanolic extracts of *Gymnema*



## Antimicrobial activity:

Organisms	Concentration of extract	Aqueous extract of <i>Gymnema</i>	Methanolic extract of <i>Gymnema</i>	Positive control (Tetracycline) (20mg/ml)	Negative control (DMSO) (20mg/ml)
<i>Escherichia coli</i> ( <i>E.coli</i> )	60mg/ml	04	16	24.30	0
	40mg/ml	--	14		
	20mg/ml	--	12		
<i>Klebsiella pneumonia</i> ( <i>K.pneumonia</i> )	60mg/ml	03	16	30.10	0
	40mg/ml	--	16		
	20mg/ml	--	12		
<i>Bacillus subtilis</i>	60mg/ml	--	14	32.24	0
	40mg/ml	--	12		
	20mg/ml	--	08		

Table 2. Zone of inhibition of leaf extracts in mm

Methanol extract exhibited good antibacterial activity with the high inhibition zones, while aqueous extract showed less activity with the concentration of 60mg/ml, 40mg/ml, 20mg/ml against *E.coli*, *Klebsiella pneumonia* and *Bacillus subtilis*. Nature is the great source to develop modern drug from medicinal plants. WHO has surveyed on significance of traditional medicine in human health care [13].

## Conclusion

Based on the above phytochemical screening and anti microbial activity results we can conclude that the plant *Gymnema sylvestre*

is a potential medicinal plant for the treatment diseases. Presence of chemical compounds in the plant, it plays an important role to cure different ailments. Further studies of principle compound isolations will be carried out.

## Acknowledgement

The authors express sincere thanks to Department of Genetics, Osmania University, Hyderabad and UGC-Engineering & Technology division for providing facilities for smooth research.

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