

## Phytochemical Screening and Antibacterial Activity of Leaf Extracts of *Gymnema sylvestre* against Pathogenic Bacteria

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**Abstract-** The aim of our present study to investigate the phytochemical screening and antibacterial activity of Ethanollic and Chloroform leaf extracts of *Gymnema sylvestre* against some important pathogenic bacteria. The Ethanollic and Chloroform leaf extracts of *G. sylvestre* showed significant antibacterial against some clinically important gram negative and gram positive bacteria such as *Staphylococcus aureus* (ATCC6538), *Enterococcus faecalis* (ATCC 29212) and *Escheria coli* (ATCC8739), *Salmonella enterica* (ATCC 10798) respectively. The in-vitro antibacterial activity was performed by agar well diffusion method (25, 50, 75 and 100 µg/ml). The maximum inhibition zone of ethanollic leaf extract was found in 100 µg/ml against gram negative bacteria such as *Salmonella enterica* (26mm), *Escheria coli* (24mm) and gram positive bacteria such as *Enterococcus faecalis* (26mm) and *Staphylococcus aureus* (24mm). The maximum inhibition zone of chloroform leaf extracts found in 100 µg/ml (25,50,75, and 100 µg/ml) against gram negative bacteria such as *Salmonella enterica* (24mm), *Escheria coli* (24mm) and gram positive bacteria *Enterococcus faecalis* (23mm) and *Staphylococcus aureus* (23mm). The phytochemical tests were carried out and showed that the antibacterial activity of plant *Gymnema sylvestre* leaves were due to presence of phytochemical compounds such as Alkaloids, Flvanoids, Glycosides, Reducing sugar, Tannins, Saponins, Polysaccharides and Phenols. The ethanollic and chloroform leaf extracts of *Gymnema sylvestre* showed maximum inhibition against *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella enterica* and *Escheriacoli*. The Ciprofloxacin was used as a positive control.

**Keywords-** *Gymnema sylvestre*, pathogenic microorganisms, antibacterial activity, bioactive components

### I. INTRODUCTION

In the recent years, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial components [1,2]. India is a paradise of medicinal plants and traditionally used many medicinal plant components are known as therapeutic properties from India [3,4]. The development of new antibacterial agent especially from the natural source is based on the pharmacological properties [5]. The *Gymnema sylvestre* is one of the most important medicinal plants that grow in tropical forests in Western India, Africa, Australia, South East Asia and India [6,7]. The plant has been reported to possess antimicrobial [8] and ethnoveterinary medicinal properties. The active ingredients are present in leaves and roots used in traditional medicine and available pharmaceutical products to treat various ailments like diabetes, asthma and etc. [9]. In the Ayurvedic system of medicine, *G. sylvestre* is referred to as

“mesarngi” and both the dried leaf and dried root are used therapeutically. The plant leaves are used in ayurvedic as antiviral, diuretic, antiallergic, hypoglycemic, hypolipidemic, for the treatment of obesity and dental caries [10]. The antioxidant and antimicrobial activities of plant extracts have formed the basis of applications in pharmaceuticals, alternative medicines and natural therapy and growing demand for *G. sylvestre* leaves in pharmaceutical trade [11]. Gymnemic acid is the active ingredient of this plant, the extracted from leaves and used widely as an anti-diabetic [12] anti-sweetner [13] and antihypercholesterolemia [14]. It has also stomachic, diuretic and cough suppressant properties [15].

The several studies are reported that leaf extract of *G. sylvestre* has protective role against gram-positive bacteria such as *S. mitis* and *S. mutans* [16] and used to treating infectious diseases caused by *Escherichia coli* and

*Staphylococcus aureus* [17] Bhuvaneshwari *et.al.*, [18] demonstrated that the methanolic extracts of *G. sylvestre* were assessed for antimicrobial activity of leaves and root separately. The results revealed that the methanol extracts in acidic range have good activity towards all the pathogens. In a similar study carried out by Satdive *et al.*, [8] the antimicrobial activity of ethanolic extracts of *G. sylvestre* against *B. subtilis*, *B. pumilus*, *S. aureus* and *P. aeruginosa* showed good antimicrobial activity. Hence, the present study was planned to find out the phytochemical properties and antimicrobial activities of ethanol and chloroform extracts of *Gymnema sylvestre*. The prepared extracts were screened for their antibacterial activity against clinically important pathogenic bacteria such as *Staphylococcus aureus*, *Enterococcus enteric*, *Escheria coli* and *Salmonella enterica*. The presence of phytochemical components and antibacterial properties can lead to detect new sources of antibacterial agents.

## II. MATERIALS AND METHODS

### Collection of plant

The leaves of *Gymnema sylvestre* were collected from Cheyyar, Thiruvannamalai District, Tamil Nadu and India. The species was identified and authenticated by the Department of Botany, Arignar Anna Government Arts College, Cheyyar, Thiruvannamalai District, Tamil Nadu.

### Microorganisms used

Gram positive bacteria such as *Staphylococcus aureus* (ATCC6538), *Enterococcus enterica* (ATCC 2912) and Gram negative organism such as *Escheria coli* (ATCC8739), *Salmonella enterica* (ATCC 10798) were used for antibacterial study.

### Preparation of plant extracts

The leaves of *Gymnema sylvestre* were cleaned and dried in shade under dark room, then cut into small pieces and coarsely powdered by mixer. The coarse powder was used for extraction with various solvents. In 1 kg of dried leaf powder added 2.5 liters of solvents (water, ethanol, and chloroform) was taken in individual aspirator bottle, were used and the mixtures were shaken occasionally in Orbital shaker for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extracts were filtered before drying using Whatmann filter paper no.1 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40°C, the extracts were placed in pre-weighed flasks before drying. Finally the aqueous, ethanol and chloroform extracts of *Gymnema sylvestre* leaves were used for the preliminary phytochemical screening and antibacterial study.

### Phytochemical Screening

Phytochemical screening in the *Gymnema sylvestre* leaf extract using different solvent systems (aqueous, ethanol

and chloroform) were carried out as per the standard method of Dey and Raman [19].

### Antibacterial Activity

Antibacterial activity of Aqueous, Ethanol and Chloroform extracts of *Gymnema sylvestre* were done by the method of Gillespe *et al.*, [20, 21] by using disc diffusion method with both Gram negative and positive bacteria.

### Procedure

Aqueous, Ethanol and Chloroform extracts (10% and 50%) solution were prepared by dissolving 1 and 5 g in 10 ml each of distilled water. 10 ml each of the prepared concentrations were pipette into sterile test tubes. Bacterial aliquots of the test organisms were made by scooping 2 colonies each of a 24 hours growth of the bacteria into 4 ml of sterile distilled water. 0.2 ml of each of the aliquots containing approximately  $5 \times 10^4$  bacterial cells or colony forming units was transferred into both of the extract concentrations and allowed to stand for an hour for reaction to take place between the extracts and the bacterial organisms. The mixtures were then inoculated on separate nutrient agar plates and incubated at 37°C for 24 hours. Ethanolic and aqueous extracts of the leaves at 10 and 50% concentration of both chloroform and aqueous extracts of leaves had inhibitory effect, hence the same concentrations were chosen as the working concentration. Results are expressed in area of inhibition zone in mm.

## III. RESULTS AND DISCUSSION

The phytochemical analysis of *Gymnema sylvestre* leaf extract using Aqueous, Ethanolic and Chloroform were showed in Table 1. From the observation of phytochemical analysis, reducing sugar was found in *Gymnema sylvestre* in all three solvents. The Ethanol and Chloroform leaf extracts of *Gymnema sylvestre* showed the presence of alkaloids, flavanoids, glycosides, tannins, saponins, polysaccharide, phenols and absence of terpenoids, phytosterols but which were present in aqueous leaf extract. Aqueous extracts of *Gymnema sylvestre* have flavanoids, glycosides, reducing sugar, polysaccharides, phytosterols and phenols. The presences of above bio active components are responsible for broad pharmacological profile, including antibacterial activity [22].

The Aqueous, Ethanol and Chloroform leaf extracts were tested using agar plate disc diffusion method against gram positive bacteria such as *Staphylococcus aureus* (ATCC6538), *Enterococcus enteric* (ATCC 2912) and gram negative bacteria such as *Escheria coli* (ATCC8739), *Salmonella enterica* (ATCC 10798. Table 2 and 3 showed antibacterial activity of ethanol leaf extract of *Gymnema sylvestre* found maximum inhibitory zone (26mm in 75 and 100µg/ml) against gram positive bacteria *Enterococcus faecalis* followed by 24mm, 25mm in 25 and 50µg/ml

respectively. The *Staphylococcus aureus*, maximum zone of inhibition (24mm in 75 and 100 µg/ml) and followed by 21mm, 23mm in 25 and 50 µg/ml respectively (Figure 1). The antibacterial activity of *Gymnema sylvestre* found maximum inhibition zone (26mm) against gram negative *Salmonella enterica* bacteria followed by 24mm, 25mm in 25 and 50µg/ml and *Escherichia coli* maximum zone of inhibition (24mm in 100 µg/ml) and followed by 19mm, 21mm and 23mm in 25, 50 and 75 µg /ml respectively (Table 4, 5 and Figure 2).

The Chloroform leaf extract of *G. sylvestre* were observed the maximum inhibition zone (23mm in 75 and 100 µg/ml) against gram positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* followed by 23mm, 23mm in 75 and 100 µg/ml respectively (Figure 3). The maximum inhibition zone of *Salmonella enterica* and *Escheria coli* were 24mm in 75 and 100 µg/ml respectively (Table 4, 5 and Figure 4).

This experimental studies explained about the presence of bioactive compounds in the *Gymnema sylvestre* leaf extract from different solvent extracts such as flavanoids, glycosides, reducing sugar, polysaccharides, phytosterols and phenols source of which compound have

been associated to antibacterial activity against pathogens [6]. The *G. sylvestre* leaf extract were used in this study of different extracts and their antibacterial efficacy depend on the extractive solvent [23]. The ethanol leaf extracts of *G. sylvestre* more potential activity than the chloroform leaf extract. The phytochemical analysis showed the presence of various bioactive compounds like alkaloids, flavanoids, glycosides, tannins, saponins, polysaccharides and phenols.

#### IV. CONCLUSION

The present studies were revealed that the ethanol and chloroform leaf extract of *Gymnema sylvestre* showed significant activity against gram positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* and gram negative bacteria *Escheria coli* and *Enterococcus faecalis*. The ethanol leaf extracts of *G. sylvestre* more potential activity than the chloroform leaf extract. The phytochemical analysis showed the presence of various bioactive compounds like alkaloids, flavanoids, glycosides, tannins, saponins, polysaccharides and phenols. This studies may further leads to the formulation of a new drug having the active compound.

**Table 1: Phytochemical screenings of leaf extract *G. sylvestre* in different solvents**

Tests	Plant extracts Aqueous	Plant extracts Ethanol	Plant extracts Chloroform
Alkaloids	-	+	+
Flavanoids	+	+	+
Glycosides	+	+	+
Reducing Sugar	+	+	+
Tannins		+	+
Saponins	-	+	+
Terpenoids	-	-	-
Polysaccharides	+	+	+
Phytosterols	+	-	-
Phenols	+	+	+

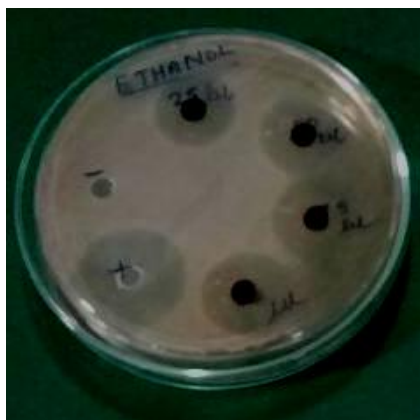
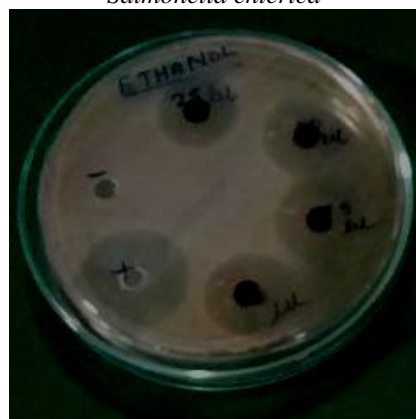
+: Present; -: Absent

**Table 2: Antibacterial activity of ethanolic extract of *Gymnema sylvestre* in Gram positive bacteria**

S.No	Name of the Micro organism	Reference Drug (Positive control)	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Negative Control
1	<i>Staphylococcus aureus</i>	26mm	21mm	23mm	24mm	24mm	-
2	<i>Enterococcus faecalis</i>	28mm	24mm	25mm	26mm	26mm	-

**Table 3: Antibacterial activity of ethanolic extract of *Gymnema sylvestre* in Gram negative bacteria**

S.No	Name of the Micro organism	Reference Drug (Positive control)	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Negative Control
1	<i>Escherichia coli</i>	26mm	19mm	21mm	23mm	24mm	-
2	<i>Salmonella enterica</i>	28mm	24mm	25mm	26mm	26mm	-

**Results for antibacterial study***Staphylococcus aureus**Enterococcus faecalis*Figure 1. Antibacterial activity of ethanolic extract of *Gymnema sylvestre* in Gram positive bacteria*Escherichia coli**Salmonella enterica*Figure 2. Antibacterial activity of ethanolic extract of *Gymnema sylvestre* in Gram negative bacteria**Table 4: Antibacterial activity of chloroform extract of *Gymnema sylvestre* in Gram positive bacteria**

S.No	Name of the Micro organism	Reference Drug (Positive control)	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Negative Control
1	<i>Staphylococcus aureus</i>	24mm	18mm	20mm	23mm	23mm	-
2	<i>Enterococcus faecalis</i>	24mm	19mm	22mm	23mm	23mm	-

**Table 5: Antibacterial activity of chloroform extract of *Gymnema sylvestre* in Gram negative bacteria**

S.No	Name of the Micro organism	Reference Drug (Positive control)	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Negative Control
1	<i>Escherichia coli</i>	30mm	20mm	22mm	24mm	24mm	-
2	<i>Salmonella enterica</i>	28mm	19mm	23mm	24mm	24mm	-

Reference Drug (Positive Control): Ciprofloxacin; Negative Control: DMSO

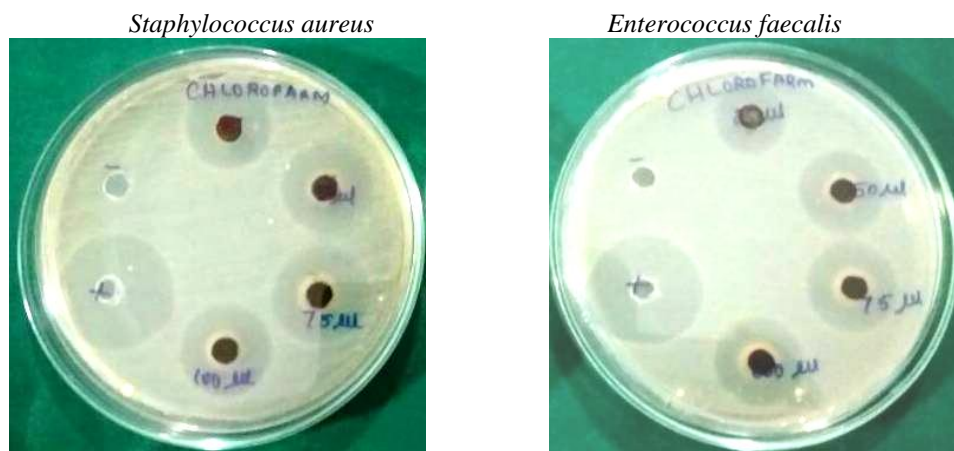


Figure 3. Antibacterial activity of chloroform extract of *Gymnema sylvestre* in Gram positive bacteria

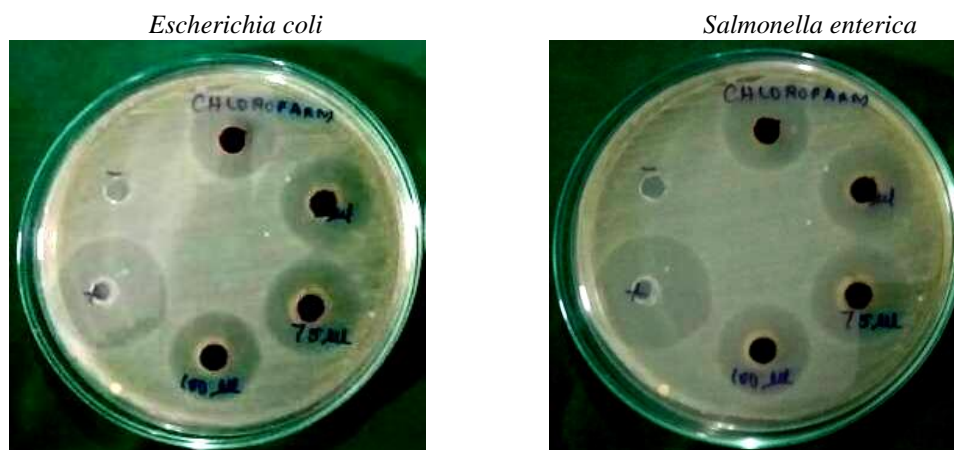


Figure 4. Antibacterial activity of chloroform extract of *Gymnema sylvestre* in Gram negative bacteria

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