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Original Research Paper

Study of the Preliminary Phytochemistry, Antibacterial and Antioxidant Activities of *Gymnema sylvestre* R. Br.

Meena Thomas Irimpan, C. I. Jolly* and D. Sheela**
Department of Botany, St. Josephs College, Irinjalakuda-680121, Distt. Trichur, Kerala, India
*R & D Department, Kerala Ayurveda Limited, Aluva-683 585, Kerala, India
**Department of Botany, St. Teresa's College, Ernakulam-682 035, Kerala, India

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ABSTRACT

The medicinal plant *Gymnema sylvestre* R. Br. is known for its antidiabetic potential. Preliminary phytochemical screening of stem and leaf samples of the herb showed positive test for flavonoids, hydrolysable tannins, phenols, saponins, sterols and terpanoids. The ash value is below the prescribed limit according to Ayurvedic Pharmacopeia. Antimicrobial activity was checked using agar well diffusion method. Water, methanol and ethanol extracts showed activity against *Staphylococcus aureus. Bacillus subtilis* is sensitive to hydroalcoholic and ethanolic extracts. The gram negative bacteria were resistant to the extracts. The antioxidant potential was assessed by DPPH assay method. The results provide evidence that *Gymnema sylvestre* leaf extract might indeed be potential source of free radical scavenger.

INTRODUCTION

Gymnema sylvestre R. Br. (Asclepiadaceae) is a vulnerable species, slow growing, perennial, medicinal woody climber found in central and peninsular India. It is a potent antidiabetic plant and used in folk, ayurvedic and homoeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, inflammation and snake bite. In addition, it possesses antimicrobial, antihypercholesterolemic, hepatoprotective and sweet suppressing activities. It also prevents dental caries caused by *Streptococcus mutans* and used in skin cosmetics (Komalavalli & Rao 2000). In Japan, there are teas being made from *Gymnema sylvestre* leaves and are being promoted as a natural method for controlling obesity and diabetes (Nakamura et al. 1999). The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites.

The rise of antibiotic resistant microorganisms is one of the severe problems in healthcare systems of the world. Therefore, it is essential to find new compounds that have antimicrobial properties and it is worthwhile to screen plant species which have the above properties to synthesize new drugs (Nascimento et al. 2000).

Oxidative stress or excessive production of reactive oxygen species (ROS) is being implicated in many diseases such as cancer, atherosclerosis, ageing, diabetes, etc. (Finkel & Holbrook 2000, Halliwell 1997, Halliwell & Gutteridge 1993). Although organisms have endogenous antioxidant defences produced during normal cell aerobic respiration against ROS, other antioxidants are taken both from natural and synthetic origin. The use of synthetic antioxidants is being restricted because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without any undesirable effect, has increased greatly (Rechner et al. 2002). Keeping all these in view the study was planned to analyse the phytochemistry, antibacterial and antioxidant activity of *Gymnema sylvestre*.

MATERIALS AND METHODS

The aerial parts of the plant *Gymnema sylvestre* R. Br. were collected, identified and authenticated. The leaf and stem were dried separately under shade, powdered and stored in closed vessel for further use. The ash value was determined as per the standard protocol given by Pharmacopoeia of India (1996). The phytochemical analysis of the extract of *Gymnema sylvestre* leaf and stem were carried out using the standard procedures (Kokate 2007).

Antimicrobial activity was checked using agar well diffusion method (Perez et al. 1990). Two Gram positive bacteria, *Staphylococcus aureus (*MTCC 3160) and *Bacillus subtilis* (MTCC 3053), and three Gram negative bacteria, *Klebsiella pneumoniae* (MTCC 3384), *Salmonella enterica typhymurium (*MTCC 98) and *Escherichia coli* (MTCC 727) were used as the test organisms. The water extract of the leaf was prepared by dissolving 2 g of leaf powder in 20 mL of

Table 1: Preliminary phytochemical screening of *Gymnema sylvestre*.

Secondary metabolite	Leaf extract		Stem extract	
	Methanol	Water	Methanol	Water
Flavonoids	+	+	+	+
Glycosides	-	-	-	-
Phenols	+	+	+	+
Saponins	-	+	-	+
Sterols	+	-	+	-
Tannins	+	+	-	-
Terpanoids	+	-	-	-

(+ Presence, - Absence)

Table 2: Ash value percentage of *Gymnema sylvestre*.

Leaf	7.09 %
Stem	4.32 %

distilled water and extracted for 20 minutes under reflux. The supernatant was filtered and the filtrate was used. Similarly 50% hydroalcoholic, ethanolic and methanolic extracts were prepared. The plates were prepared by using Muller Hinton agar (Hi-Media). Eighteen hour old culture of test organisms in Nutrient Broth was used as inoculum. 150 µL each of the extract was used. Gentamycin disc (10 mcg/disc) was used as the positive control. The diameter of zone of inhibition was measured after an incubation period of 24 hours at 37°C.

Free radical scavenging activity was determined by DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay method (Brand-Williams et al. 1995). The method is based on the reduction of a methanolic solution of the coloured free radical DPPH by free radical scavenger. The decrease in absorbance of DPPH at its absorbance maximum of 516 nm is proportional to the concentration of free radical scavenger (methanolic extract of *Gymnema sylvestre* leaf) added to the DPPH reagent solution. The activity is expressed as IC_{50} (i.e. the concentration of the test solution required to give 50% decrease in absorbance compared to that of a control solution). IC_{50} was calculated by plotting the linear regression calibration curve of log of concentration of test solution on X-axis against percentage reduction in absorbance on Y-axis. Ascorbic acid pure was used as a standard.

RESULTS AND DISCUSSION

The medicinal value of a plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga et al. 2005). The leaf extract shows the presence of flavonoids, phenols, saponins, sterols, tannins and terpanoids, and the stem extract contains flavonoids, phenols, saponins and sterols (Table 1). Determination of ash values are helpful in determining the quality and purity of crude drugs in powdered form. According to Ayurvedic Pharmacopeia volume of the ash value of *Gymnema sylvestre* should not exceed 12%, which is below the prescribed limit (Table 2).

The different extracts showed antibacterial activity against Gram positive bacteria *Staphylococcus aureus* and

Table 3: Antibacterial activity of leaf extracts of *Gymnema sylvestre*.

Name of bacteria	Diameter of zone of inhibition in millimeters				
	Water extract	Methanol extract	Hydroalcoholic extract	Ethanolic extract	Gentamycin
Gram positive bacteria					
Staphylococcus aureus	14	17	-	14	21
Bacillus subtilis	-	-	15	12	26
Gram negative bacteria					
Klebsiella pneumoniae	-	-	-	-	20
Salmonella typhymurium	-	-	-	-	20
Escherichia coli	-	-	-	-	28

(- No zone of inhibition)

Table 4: Antioxidant activity of *Gymnema sylvestre*.

Concentration (µg/mL)	Absorbance at 516 nm	Log concentration	Percentage inhibition %
Control	0.832	-	-
154.025	0.517	2.1875	37.8605
308.05	0.271	2.4886	67.4278
770.125	0.146	2.8866	82.4519

IC_{50} value of *Gymnema sylvestre* leaf methanol extract is 206.5 µg/mL; IC_{50} value of ascorbic acid is 8.91µg/mL.

Bacillus subtilis (Table 3). The secondary metabolites present in the plant could be responsible for some of the observed antimicrobial activity. The hypoglycemic activity of *Gymnema sylvestre* (Nakamura et al. 1999) coupled with its antibacterial activity is a positive development because, diabetic patients are at a high risk of *Staphylococcus aureus* infections and this could be used to alleviate skin and pulmonary infections which are more frequent in them.

In recent years much attention has been devoted to natural antioxidants and their association with health benefits (Ali et al. 2008). In this study, the free radical scavenging activity of methanol extract of *Gymnema sylvestre* leaf was found to be dose dependent. IC_{50} value of *Gymnema sylvestre* leaf methanol extract was 206.5 µg/mL (Table 4). Though the DPPH radical scavenging abilities of the extract were less than that of pure ascorbic acid, the study showed that it could serve as free radical inhibitor or scavenger, acting possibly as primary antioxidant.

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