Research Article

Screening of Methanolic Extract of *Gymnema Sylvestre* R. Br. leaves for Antioxidant Potential

Laxmi Ahirwal*[1], Siddhartha Singh[2], Subhadip Hajra[1], Archana Mehta[1]

[1]Lab of Plant Pathology and Biotechnology, Department of Botany, School of Biological and Chemical Sciences, Dr. H.S. Gour Central University, Sagar, M.P., India.

[2] Lab of Biotechnology, Noble College, Rajakhedi, Sagar, M.P., India.

***Corresponding author's E-mail:** laxmirays@gmail.com

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ABSTRACT

The present study was undertaken to evaluate the phytochemicals presence, total phenolic contents and *in vitro* antioxidant potential of methanolic extract of *Gymnema sylvestre* R. Br. leaves. *Gymnema sylvestre* commonly known as gudmar belongs to the family Asclepiadaceae. *In vitro* antioxidant potential was evaluated by using DPPH, hydroxyl radical, nitric oxide radical scavenging as well as ferric reducing power assays. The extract showed significant activities in a concentration dependent manner when compared with standard drug Butylated hydroxy anisole. The total phenolic content in methanolic extract of *G. sylvestre* leaves was 2.2 µg/mg of Catechol equivalent. The IC_{50} value of the DPPH method, hydroxyl radical and nitric oxide radical scavenging activity was 450, 625.33, 1723.96 µg/ml respectively. In ferric reducing power methanolic extract of *G. sylvestre* showed absorbance 0.13 at 500 µg/ml. Results revealed that the methanolic extract of *G. sylvestre* showed significant antioxidant activity. Phytochemical screening showed the presence of phenolic compound such as flavonoids, tannins and saponins which may be responsible for the activity. The results suggest that extract may act as a natural antioxidant agent and offering effective protection from free radicals.

Keywords: Antioxidant activity, *Gymnema sylvestre*, Methanolic extract, Total phenolic content.

INTRODUCTION

Reactive oxygen species (ROS) are various forms of activated oxygen, which include free radicals such as superoxide ion (O_2^-) and hydroxyl radicals (OHO), as well as non free- radical species such as hydrogen peroxide (H_2O_2)[1,2]. Reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer[3-6]. The scavenger of free radical is known as antioxidant. Synthetic antioxidants have been widely used industrially to control lipid oxidation in foods; however, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity[7]. The search for antioxidants from natural sources has received much attention and efforts have been put into identify compounds that can act as suitable antioxidants to replace synthetic ones. Medicinal plants are a source for a wide variety of natural antioxidants[8].

Gymnema sylvestre R. Br. commonly known as gudmar belongs to the family Asclepiadaceae. It is a woody, climbing herb grown in India, China, Indonesia, Japan, Malaysia, Srilanka, Vietnam and South Africa[9]. The plant is useful in inflammations, hepatosplenomegaly, dyspepsia, constipation, haemorrhoids, helminthiasis, cough, asthma, bronchitis, cardiopathy, jaundice, intermittent fever, piles, amenorrhea, conjunctivitis, leucoderma, and urinary disorders and leaves (triturated and mixed with castor oil) are applied to swollen glands and enlargement of internal viscera as the liver and spleen[10]. Its root is used to cure snakebite. The fresh leaves when chewed have the remarkable property of paralysing the sense of taste for sweet and bitter substance for some time[11]. According to Ayurvedic literature leaves were used as a cardiotonic, diuretic[12], laxative, stimulant, stomachic and uterine tonic and these are also used as antiviral, diuretic, antiallergic, hypoglycemic, hypolipidemic, for the treatment of obesity and dental caries[13]. It is a potent antidiabetic plant used in folk, ayurvedic and homeopathic systems of medicine. It is reported to possess antimicrobial, antihypercholesterolemic, hepatoprotective, anti-allergic, antiviral, lipid lowering effects[14]. On the basis of above background the present study was undertaken for the evaluation of *in vitro* antioxidant activity.

MATERIALS AND METHODS

Plant material

The leaves of *Gymnema sylvestre* were collected from Jeevan herbs Agro, Sagar (M.P.) and authenticated at the Department of Botany, Dr. H. S. Gour University, Sagar. The identification of the plant materials was done by Taxonomist and Herbarium incharge (specimen number Bot/Her/B/1314), Department of Botany, Dr. H.S. Gour University, Sagar (M.P.).

Extract preparation

Dried and powdered plant material (60 g) was defatted with petroleum ether (500 ml) and then successively extracted with methanol (500 ml at 40˚C) using soxhlet apparatus. The extract was cooled at room temperature, filtered and evaporated to complete dryness. The percentage yield of the extract was calculated and it was found to be 5.77%.

Phytochemical analysis

The methanolic extract was subjected to various chemical tests to detect the presence of various phytochemicals such as tannins, flavonoids, alkaloids etc. using standard procedure[15,16].

Antioxidant assay

Determination of total phenolic content

Aliquots of 0.1 to 1.0ml of each sample were pipette out in a series of test tubes and the volume was made up to 3ml with distilled water. 0.5ml of Folin-Ciocalteau reagent was added to each tube and incubated for 3 min at room temperature. Sodium carbonate (20%; 2ml) solution was added, mixed thoroughly and the tubes were incubated for 1 min in boiling water bath. Absorbance was measured at 650nm against a reagent blank. Standard curve using different concentrations of standard phenolic catechol was prepared. From the standard curve, concentration of phenols in the test samples was determined and expressed as µg of catechol equivalent[17].

Ferric reducing power activity

Various concentrations of extracts (100, 250 and 500 µg) were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferric-cyanide. The mixture was incubated at 50ºC for 20 min then 2.5 ml of 10% trichloroacetic acid (w/v) were added. 5 ml of this solution was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxy anisole (BHA) was used as standard antioxidant[18].

Free radical scavenging activity by DPPH method

Different concentrations (50, 100, 250 and 500 µg) of extract and BHA were taken in different test tubes. The volume was adjusted to 500 µl by adding methanol. 5ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at room temperature for 20 min. The absorbance of the samples was measured at 517 nm[19]. Radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Hydroxyl radical scavenging activity

Various concentrations (50, 100, 250 and 500 µg) of extract was taken in different test tubes and made up to 250µl with 0.1M phosphate buffer. 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%) and 1 ml of Dimethyl sulphoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. These reaction mixtures were incubated at room temperature for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). 3 ml of Nash reagent (150 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for colour development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank[19]. The percentage hydroxyl radical scavenging activity was calculated by the following formula:

$$\% \text{ hydroxyl radical scavenging activity} = \frac{1 - \text{Difference in absorbance of sample}}{\text{Difference in absorbance of blank}} \times 100$$

Nitric oxide radical scavenging activity

Various concentrations (50, 100, 250 and 500 µg) of extracts were taken in different test tubes and made up to 3 ml with 0.1M phosphate buffer (pH 7.2). Sodium Nitroprusside (5 mM) prepared in buffered saline (p^H7.2) was added (1 ml) to each tube. The reaction mixture was incubated for 30 min at RT.

A control without the test compound, but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1- Naphthylethylenediamine dihydrochloride). The absorbance of the samples was measured at 546 nm[20]. Nitric oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Table 1: Phytochemical analysis of methanol extract of *G. sylvestre* leaves

Phytochemical	Methanol Extract
Steroids	-
Terpenoids	-
Tannins	+
Alkaloids	+
Glycosides	+
Flavonoids	+
Saponins	+

+: Present; -: Absent

Table 2: Total phenolic contents of catechol equivalent in methanol extract of *G. sylvestre* leaves

Test Sample	µg/mg of catechol equivalent phenolics
GSM	2.2

GSM = *Gymnema sylvestre* methanol extract

RESULTS AND DISCUSSION

Total phenolic content

The total phenolic content in methanolic extract of *G. sylvestre* leaves was 2.2 µg/mg of Catechol equivalent (Table 2). Phenolics are the most wide spread secondary metabolites in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant. The antioxidant activity of the plant extract is mainly due to presence of phenolic compounds due to their redox properties, hydrogen donors and singlet oxygen quenchers[21]. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food[22].

Free radical scavenging activity by DPPH method

The free radical-scavenging activity of the extract was measured in terms of hydrogen donating or radical-scavenging ability[19]. The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay which is commonly employed for screening of plant extracts was used for hydrogen donating capacity[23]. The results were reported in terms of IC_{50} values. The IC_{50} value of the methanolic extract of *G. sylvestre* was 450 µg/ml. The result was compared with the IC_{50} value of BHA (152.74 µg/ml). Extract showed potent DPPH radical scavenging activity (3.4, 12, 22.5 and 54.54%) at the concentration of 50, 100, 250 and 500 µg/ml respectively (Table 3; Figure 1). The extract showed concentration dependent DPPH scavenging activity when compared with that of standard drug butylated hydroxy anisole (BHA).

The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability. The plants with higher donating capacity have shown higher DPPH free radical scavenging activity[24].

Hydroxyl radical scavenging activity

The hydroxyl radicals scavenging activity (50, 100, 250 and 500 µg/ml) was analyzed and the results were reported in terms of IC_{50} values. IC_{50} values of methanolic extract of *G. sylvestre* and BHA was found 625.33, and 335.43 µg/ml respectively. The percentage inhibition of methanolic extract of *G. sylvestre* on hydroxyl radical scavenging was 1.43, 3.81, 17.62 and 40.95% at the concentration of 50, 100, 250 and 500 µg/ml respectively (Table 3; figure 2).

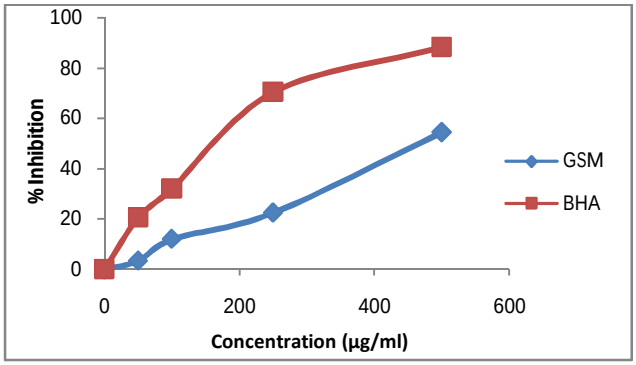

Figure 1: Effect of methanolic extract of *G. sylvestre* on free radical scavenging activity by DPPH method

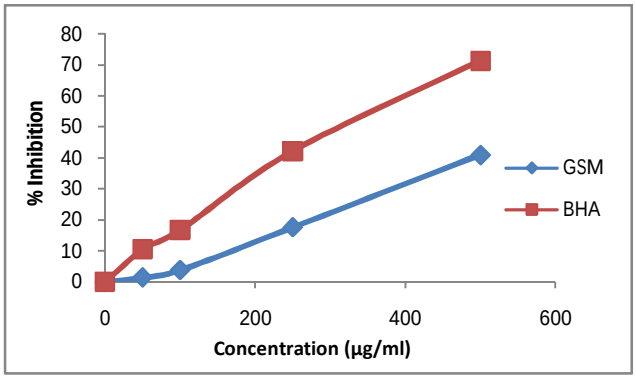

Figure 2: Effect of methanolic extract of *G. sylvestre* on hydroxyl radical scavenging activity

Table 3: Effect of *G. sylvestre* methanol extract on different radical-scavenging activities

Concentration (µg)	Free radical scavenging activity by DPPH method		hydroxyl radical scavenging activity		nitric oxide radical scavenging activity		Ferric reducing power activity	
	GSM	BHA	GSM	BHA	GSM	BHA	GSM	BHA
50	3.4±0.23	20.7±0.16	1.43±0.24	10.56±0.12	11.4±0.11	9.7±0.21		
100	12±0.12	32.2±0.14	3.81±0.26	16.77±0.14	13.3±0.13	17.1±0.10	0.01±0.22	0.42±0.23
250	22.5±0.24	70.5±0.23	17.62±0.12	42.24±0.28	15.2±0.17	20.5±0.12	0.051±0.24	0.96±0.25
500	54.54±0.21	88.4±0.03	40.95±0.17	71.34±0.21	17.7±0.23	26.7±0.14	0.13±0.12	1.74*±0.18
IC_{50}	450.26±0.13	152.74±0.14	625.33±0.20	335.43±0.21	1723.96±0.25	962.6±0.16		

GSM= *Gymnema sylvestre* methanol extract; BHA= Butylated hydroxy anisole; *= absorbance beyond the limit

The ability of the extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and scavenger of active oxygen species, thus reducing the rate of the chain reaction. Ethanolic extract of *Stevia rebaudiana* leaves also showed similar hydroxyl radical

scavenging activity in a concentration dependent manner[25].

Nitric oxide radical scavenging

The nitric oxide radical scavenging activity of different extract at different concentration (50, 100, 250, 500 µg/ml) were analyzed in terms of IC_{50} value. IC_{50} value of methanolic extract of *G. sylvestre* was found 1723.96 and 962.66 µg/ml respectively. Methanolic extract of *G. sylvestre* showed 11.4, 13.3, 15.2 and 17.7 % inhibition at 50, 100, 250, 500 µg/ml respectively (Table 3; Figure 3). The percentage inhibition of the extract was compared with BHA. The above results revealed that methanolic extract of *G. sylvestre* showed significant antioxidant activity.

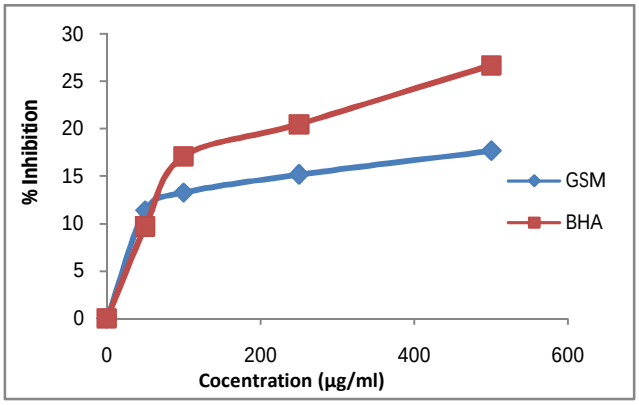

Figure 3: Effect of methanolic extract of *G. sylvestre* on Nitric oxide radical scavenging activity

Nitric oxide is an essential bio-regulatory molecule required for several physiological processes like neural signal transmission, immune response, control vasodilatation, control of blood pressure etc[26,27]. Nitric oxide is lipophilic in nature and easily diffuse between cells. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide[28]. Nitric oxide plays an important role in various types of inflammatory processes in the animal body. The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation.

Ferric reducing power

The ferric reducing power of different extracts and standard drug was analyzed by testing their absorbance at 700nm. The various concentrations (100, 250 and 500 µg/ml) of methanolic extract of *G. sylvestre* and BHA showed absorbance 0.01, 0.051, 0.13 and 0.42, 0.96, 1.74 respectively (Table 3; Figure 4). The reducing power of methanolic extract of *G. sylvestre* (Fe^{3+} – Fe^{2+}) was found to be increased with increasing the concentration. The result obtained in the present studies showed that extracts consist of hydrophilic poly phenolic compounds that cause the reducing power.

The antioxidant activity of phenolic compounds is mainly due to its redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[29]. Tanaka *et al.,* (1988) have also observed a direct correlation between antioxidant activity and reducing power of certain plant extracts[30]. The reducing properties are generally associated with the presence of reductones[31], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[32]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

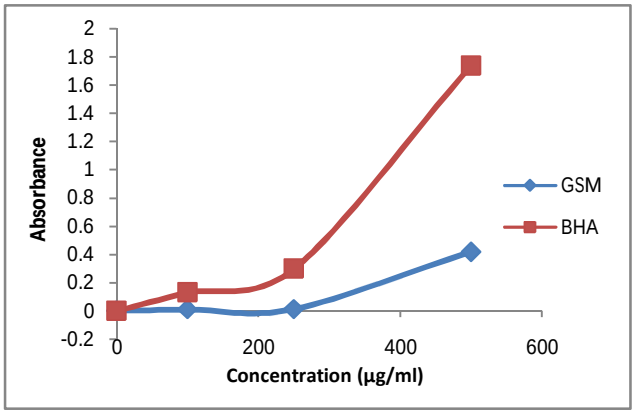

Figure 4: Effect of methanolic extract of *G. sylvestre* on ferric reducing power

Similarly, antioxidant potential of the methanol extract of the leaves of *Mimusops elengi* was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, reducing power and total antioxidant capacity. The extract showed significant activities in all antioxidant assays compared to ascorbic acid (standard drug) in a dose dependent manner. In DPPH scavenging assay the IC_{50} value of the extract was found to be 43.26µg/ml, while the IC_{50} value of the standard drug (ascorbic acid) was 58.92 µg/ml. Total antioxidant activity was also found to increase in a dose dependent manner[24].

The above result revealed that the methanolic extract of *G. sylvestre* showed significant antioxidant activity. In phytochemical tests, in *G. sylvestre*, methanolic extract revealed the presence of alkaloids, tannins, saponins, flavonoids, glycosides and carbohydrates (Table 1). So antioxidant activity shown by methanolic extract of *G. sylvestre* leaves might be due to presence of these phytochemicals. Thus, it can be concluded that methanolic extracts of *G. sylvestre* leaves can be used as an accessible source of natural antioxidants agent with consequent health benefits.

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