



## Physico-chemical composition, Phytochemical analysis and in vitro Antioxidant Activity of *Gymnema Sylvestre* root

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### ABSTRACT

The *Gymnema sylvestre* plant which is known to its variety of medicinal value in the folk medicine. The present study reports Moisture Content, Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble Extractive value of root of *Gymnema sylvestre*. The phytochemical analysis of *G. Sylvestre* using water extract indicates the presence of phytochemicals like phenolics, saponins and flavonoids. Water extract of *Gymnema sylvestre* root was selected to evaluate antioxidant potential using different in vitro antioxidant assay procedure. The results of present study showed that the root of the plant contains antioxidant compounds. The total phenolic content of water extract of *G. Sylvestre* Root is  $97 \pm 0.98$  mg/GAE dry weight. The water extract of root shows good DPPH radical scavenging activity. The reducing and Beta carotene bleaching activity was moderate.

**Keywords:** Phytochemical analysis, Physico-chemical analysis, antioxidant activity, *G. sylvestre*

### INTRODUCTION

*Gymnema sylvestre* R.Br. (Family: Asclepiadaceae), commonly known as 'Gurmar', is a well-known indigenous medicinal plant used in the treatment of diabetes and many other ailments. The plant is woody climber, located in central and western India, Tropical Africa and Australia. It is commonly distributed in Western Ghats of Maharashtra in India. The leaves of the plant have unique property to inhibit sweet taste [1]. A research articles on *Gymnema sylvestre* shows that leaves of this plant contains variety of biologically active components. The active compound of the plant is a group of acid termed as gymnemic acid [2].

A recent review describes the antimicrobial, hepatoprotective, antihypercholesterolemic and anti-inflammatory activities of leaves of this plant [3, 4]. They are used for making antidiabetic formulations in folk, ayurvedic and homeopathic medicines. The *G. Sylvestre* also contains essential oil having antioxidant and antimicrobial activity [5]. In the present study, proximate analysis, phytochemical analysis and antioxidant activity of root extract of *G. sylvestre* was determined by using DPPH,  $\beta$ -carotene bleaching and ABTS radical scavenging assays.

### RESEARCH METHODS

#### Plant material

The plant of *G. sylvestre* (2 Kg) was collected from 'Pune' (Mulashi) from Maharashtra state in India. The plant was authenticated by Botanical Survey of India, Pune (BSI). The material has been deposited at AHMA herbarium at BSI (Voucher No.SVS-1/7).

#### Chemicals

Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), potassium ferricyanide, trichloroacetic acid, ferric chloride and tween-20 were purchased from Loba Chemicals, linoleic acid was purchased from SRL,  $\beta$ -carotene from HIMEDIA and Folin-Ciocalteu reagent was purchased from Qualigens. These are Indian companies. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azinobis-(3-ethyl benzothiazoline-6-sulphonic acid) diammonium salt (ABTS), ammonium persulphate, were purchased from Fluka, USA. All the solvents used were of analytical grade.

**Proximate Analysis**

The Physico-chemical analysis of *G. Sylvestre* is carried out by using procedure as described in [6].

**Preparation of extract**

Dried and powdered root (500 g) of *G. sylvestre* was subjected to cold extraction with n-hexane (1.5 lit) at room temperature (4 x 16 h). The dried powder was then extracted with distilled water (1.5 lit) followed water: ethanol (1:1) at room temperature. The combined water extract was concentrated under reduced pressure at 60°C to one third of its volume. The combined extract was subjected for further study.

**Phytochemical Analysis**

The phytochemical Screening of the extracts was done using standard procedure as described in [7, 8, 9]. The following qualitative tests were carried out as follows.

**Steroids:** 10 mg 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 presence of terpenoids.

**Terpenoids:** To 0.5 gram of plant extract was added to 2 ml chloroform. Concentrated sulphuric acid (3 ml) was added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoids.

**Alkaloids:** The aqueous extract was heated on a boiling water bath with 2 % Hydrochloric acid. After cooling the mixture was filtered and treated with a few drops of Mayer's reagent. The sample was observed for turbidity or yellow precipitation.

**Flavonoids:** The 4 ml of extract was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloric acid was added and red colour was observed for flavonoids and orange color for Flavones.

**Coumarins:** 10 gram 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 Coumarins.

**Saponins:** To 0.5 gram of extract was boiled in 10 ml water in test tube. The solution was shaken vigorously and observed for persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, after which it was observed for the formation of emulsion.

**Tannins:** About d and observed for brownish green or a blue black colouration. 0.5 gram of the extract was boiled in 10 ml water in test tube and the filtered. A few drop of 0.1 % Ferric Chloride was added.

**Phenolic Compounds:** Extract was dissolved in alcohol and 1 drop of neutral ferric chloride was added to this. The intense colour indicated the presence of phenolic compounds.

**Anthraquinones:** To the extract Magnesium acetate solution was added the pink color developed indicates the presence of Anthraquinones.

**Quinone:** Few mg of the Extract in alcohol is treated with sulphuric acid. The color developed indicates the presence of quinones.

**Catechin:** Few drops of extract are treated with a few drops of Ehrlich reagent and few drops of Concentrated HCl. The pink colour developed indicates the presence of Catechin.

**Reducing Sugar:**

Aqueous extract was added to boiling Fehling's solution (A and B). The solution was observed for a colour of Reaction.

**Antioxidant assay****Determination of free radical scavenging activity (DPPH)**

The standard protocol of DPPH assay [10] was followed with slight modifications. Solutions of different concentrations of samples with standard, BHT, (20, 40, 60, 100, 200, 300, 400 µg/ml) in methanol were prepared. To the test solution (1 ml), DPPH solution (0.1mM, 1 ml) in methanol was added. Total volume was made up to 4 ml using methanol. After 30 minutes incubation in the dark, absorbance was recorded at 515 nm. The percentage of inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{[A_c - (A_t - A_b)]}{A_c} \times 100$$

Where,  $A_c$  = absorbance of control,  $A_t$  = absorbance of test solutions/standard,  $A_b$  = absorbance of blank solution.

Antioxidant activity of the samples is expressed as  $IC_{50}$  values. The  $IC_{50}$  value is defined as the concentration of sample which inhibits 50 % of DPPH radical. All the experiments were performed in triplicate.

#### **Determination of antioxidant activity using Beta-carotene bleaching assay**

Antioxidant activity was measured using standard protocol [11] with slight modifications. To 3.34 mg of Beta-carotene in chloroform solution (1 ml), 40 mg linoleic acid and 400 mg Tween-20 were added. The chloroform was then removed at 40°C under vacuum using a rotary evaporator. The resulting mixture was diluted with 10 ml distilled water and was mixed well. The emulsion was further made up to 100 ml with 0.01M hydrogen peroxide. The test solution of different concentrations (500 µg/ml and 1000 µg/ml) of each sample and the standard solutions of BHA and BHT (100 µg /ml) in methanol were prepared. Aliquots (2 ml) of emulsion were transferred into different test tubes containing 0.1 ml of test samples and standards in methanol. A control containing 0.2 ml methanol and 4 ml of the above emulsion was prepared. In this experiment BHT was used as standard. The test tubes were placed in water bath at 50°C. Absorbance of all the samples at 470 nm were taken at zero time and after every 15 mins till the colour of β-carotene disappeared in the control. The blank was prepared as described above without β-carotene. The % inhibition was determined by the following equation:

$$\% \text{ Inhibition} = \frac{(A_{A(90)} - A_{C(90)})}{(A_{C(0)} - A_{C(90)})} \times 100$$

Where,  $A_{A(90)}$  is the absorbance of antioxidants at 90 min.,  $A_{C(90)}$  is the absorbance of control at 90 min.,  $A_{C(0)}$  is the absorbance of control at 0 min. All the experiments were performed in triplicate.

#### **Reducing Power assay**

This was carried out as described previously [12]. The test solution of different concentrations (200 µg /ml, 400 µg /ml, 600 µg /ml and 800 µg /ml) and the standard solution BHT (100 µg /ml) in methanol were prepared. Solutions of plant extract was mixed with 2.5 ml phosphate buffer (0.2M, PH=6.6) and 2.5 ml potassium ferricyanide  $K_3FeCN_6$  (10g/ml). Then mixture was incubated at 50°C for 30 mins. 2.5 ml solution of Trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000rpm for 10 mins. Finally, 2.5ml of supernatant solution was mixed with 2.5 ml  $FeCl_3$  and absorbance measured at 700 nm. BHT was used as standard. Increased absorbance of the reaction mixture indicates stronger reducing power. All the samples were analyzed in triplicate.

#### **Total Phenolic content**

The total phenolic content was determined by the reported method [13] using Folin-Ciocalteu reagent. A solution of the sample of concentration 100 µg/ml in methanol was prepared. To 1 ml of this solution, 1 ml Folin-Ciocalteu reagent was added. After 5 min. 10 ml of  $Na_2CO_3$  (7%) was added to the mixture. This solution was diluted to 25 ml

using distilled water. After incubation for 90 min. at room temperature, the absorbance against reagent blank was determined at 750 nm. Total Phenolic content of the samples were expressed as mg gallic acid equivalent (GAE) / 1 g. All the experiments were performed in triplicates.

## RESULTS AND DISCUSSION

### Physico-chemical analysis

Physico-chemical analysis *G. Sylvestre* is used to assess their potential nutritive and medicinal benefits. The Physico-chemical analysis of *G. Sylvestre* root extract revealed that the presence of foreign organic matter, Acid insoluble, Water soluble ash, Ethanol & water soluble extractives, moisture content and total ash. The results are presented in Table No. 1.

Table 1: Physico-chemical Analysis of *G. Sylvestre* root.

Sr. No	1	2	3	4	5	6	7	8
Parameter	Foreign organic matter	Ethanol soluble extractive	Water soluble extractive	Total ash content	Acid-insoluble ash	Water soluble ash	Loss on drying	Moisture content
Root	3.50± 0.05%	7.60± 0.03%	3.54± 0.02%	0.040± 0.04%	0.015± 0.02%	0.54± 0.02%	5.2± 0.05%	5.33± 0.06%

Values are presented as mean ± standard deviation

### Phytochemical Analysis

The preliminary phytochemical screening study of *G. Sylvestre* root extract revealed the presence of alkaloids anthraquinones, Flavonoids, Phenols, Steroids, Tannins and Terpenoids. The therapeutic effect of medicinal plant is due presence of these secondary products present in the plant. Phytochemical screening of *G. Sylvestre* root indicates that the plant is richest source of Phytochemicals like saponins, glycosides, tannins and flavonoids. Saponins and glycosides were found in higher concentrations but lower concentration of phenols, flavonoids, alkaloids, steroids were recorded. The results are presented in Table No.2.

Table 2: Phytochemical analysis of *G. sylvestre* Root Extract.

Sr. No.	Phytochemicals	Water Extract
1	Tannins	-
2	Flavonoids	+
3	Terpenoids	+
4	Saponins	+
5	Steroids	-
6	Carbohydrates	+
7	Glycosides	+
8	Anthraquinones	-
9	Anthocyanins	-
10	Phenolics	+

+ = Present and - = Absent

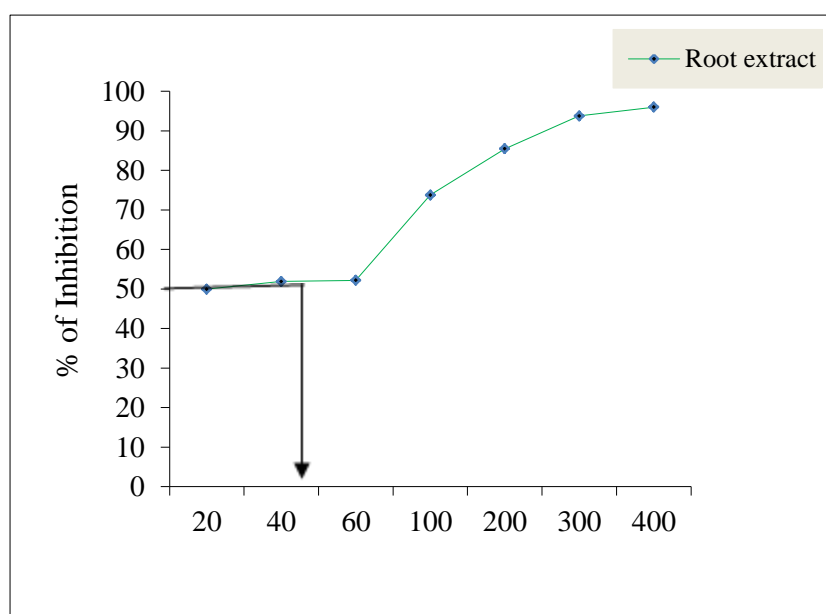
Table 3: Antioxidant Activity of *G. Sylvestre* root.

Sr. No	Parameter	Water Extract	BHT
1	Yield (%)	8.48±0.12%	-
2	Phenolic content (mg/GAE dry weight)	97.0± 0.98	-
3	IC <sub>50</sub> (mg/ml)	52.1±0.20	20

Values are presented as mean ± standard deviation

### Antioxidant Activity

Free radicals or reactive oxygen species (ROS) are formed in our body as a result of biological oxidation. The reactive oxygen species like hydroxyl radical, super oxide anion radical, hydrogen peroxide are scavenged by endogenous defense systems such as catalase, superoxide dismutase and peroxidase-glutathione. But these systems may not be completely efficient requiring them to depend on exogenous anti-oxidants from natural sources. The medicinal plants having antioxidant potential can be used as exogenous anti-oxidants. DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. Figure 1 shows IC<sub>50</sub> of extracts and BHT. The DPPH radicle scavenging activity of standard BHT was greater than that of water extract of *G. Sylvestre* root. The IC<sub>50</sub> value for the extract was found to be 52.1±0.20 µg/mL.

Figure 1: IC<sub>50</sub> of Root Extract of *G. Sylvestre*.

In linoleic acid-β-carotene bleaching method, oxidation of linoleic acid was significantly inhibited by water extract at both the concentrations, i.e. 100µg/ml and 500µg/ml [Figure 2]. At 100 µg/ml % inhibition was 81.75±0.04. At 500 µg/ml, % inhibition was 93.91±0.02 which is higher than that of standard BHT. The results are presented in Table 4.

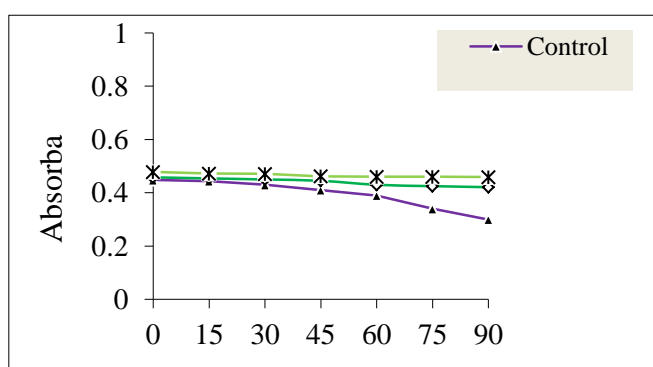
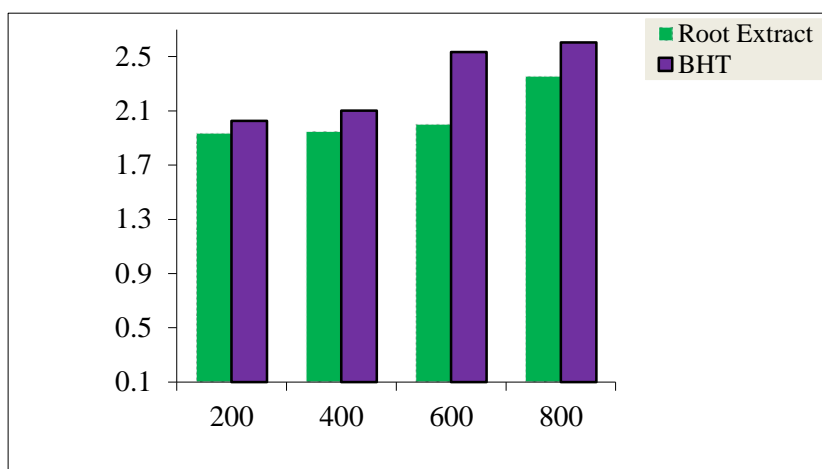


Figure 2: Beta Carotene Bleaching Assay of Root Extract of *G. sylvestre*.Figure 3: Reducing Capacity of Root Extract of *G. sylvestre* and BHT.Table 4: % Inhibition of *G. Sylvestre* root in  $\beta$ -Carotene-Linoleic acid.

Sr. No.	Compound/ Extract	$\beta$ -Carotene-Linoleic acid assay % Inhibition	
		100 $\mu$ g	500 $\mu$ g
1.	BHT	84.01 $\pm$ 0.06	80.8 $\pm$ 0.22
2.	Root Water Extract	81.75 $\pm$ 0.04	93.91 $\pm$ 0.02

Values are presented as mean  $\pm$  standard deviation

The reducing activity of water extract was determined by ferric reducing assay. Figure 3 indicates that reducing capacity was found to be concentration dependent. Reducing power of extract was lesser when compared to BHT.

### Total Phenolic Content

The amount of total phenols in the root extract was estimated by the Folin-Ciocalteu method. The content of total phenols is expressed as GAE. The phenolic content of root water extract was found to be 97 $\pm$ 0.98  $\mu$ g GAE/mg extract.

### CONCLUSION

In the present study physiochemical analysis, phytochemical analysis, total phenolic content and antioxidant capacity. *G. Sylvestre* plant was studied. The observed antioxidant activity could be related to the presence of appreciable amount of phenolic contents in the extract. To the best of our knowledge, this is the first report on antioxidant activity of root extract of *G. Sylvestre*.

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