

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITIES OF *GYMNEMA SYLVESTRE* R. BR. STEM AND LEAF EXTRACTS

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ABSTRACT

Objective: This study of aims to analyze Phytochemical analysis from *Gymnema sylvestre* leaf and stem extract and their antioxidant capability.**Methods:** The methanol extract was subjected to both qualitative and quantitative evaluations utilizing standard protocols. Fresh plant leaf extracts are used for qualitative phytochemical analysis to find the presence of the different phytoconstituents. High-performance liquid chromatography was used for quantitative phytochemical analysis, where as the 1,1-diphenyl-2-picryl hydrazine (DPPH)-free radical technique was used for antioxidant activity. It has been demonstrated that the phytochemical components of medicinal plants are mostly responsible for their antioxidant qualities.**Results:** Antioxidant activity of methanolic leaf extract of *Gymnema sylvestre* R.Br. Might be due to the presence of Cardiac glycoside, Phenols, Saponins, Glycoside, found in the preliminary phytochemical analysis. The dried leaves were extracted under continuous hot extraction in soxhlet apparatus with 90% methanol gave the gymnemic acid (0.02 %), Gymnemagenin (0.61%), Deacyl gymnemic acid (2.50%) by using HPLC methods. Percentage of Inhibition increases with increasing concentration, showing free radical scavenging activity. At 1000 µg/ml, GSM shows 82.97% inhibition, while ascorbic acid shows 89.08% inhibition at 100µg/ml.**Conclusion:** *G. sylvestre* leaf methanol extract exhibits strong antioxidant properties.**Keywords:** *Gymnema sylvestre*, High-performance liquid chromatography, 1,1-diphenyl-2-picryl hydrazine assay, Antioxidant.© 2025 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2025v18i8.54874>. Journal homepage: <https://innovareacademics.in/journals/index.php/ajpcr>

INTRODUCTION

A native medicinal herb, *Gymnema sylvestre* is a member of the *Asclepiadaceae* family, dicotyledonous class [1]. It has a long history and is one of the main botanicals used in the Ayurvedic medical system to treat ailments such as snake bites, diabetes, and malaria [2]. The purpose of this study was to evaluate the methanol extract of *G. sylvestre* phytochemical screening, antioxidant, and free radical scavenging properties. In a Soxhlet apparatus, the leaves were continuously heated to extract 90% methanol. High-performance liquid chromatography (HPLC) analysis, a preparative chromatographic technique, was used to purify gymnemic acid [3]. These essential compounds, known as antioxidants, have the capacity to shield the body from oxidative stress brought on by free radicals [4]. Free radicals are produced during all of the metabolic processes that take place within cells. However, these free radicals are efficiently neutralized by a number of antioxidant defense systems, and the body preserves the balance between oxidation and anti-oxidation [5]. It is believed that oxidative stress is the main cause of many human illnesses. Damage to DNA and oxidation of macromolecules such as proteins and lipids are thought to be the main causes of many serious illnesses [6]. Antioxidants are substances that scavenge reactive oxygen species (ROS) or lower their formation, protecting cellular constituents from their harmful effects [7]. Natural sources are the primary source of dietary antioxidants. Furthermore, researchers are paying more attention to plant-derived antioxidants because synthetic antioxidants have been linked to cancer and detrimental effects on the liver and lungs [8]. Natural sources of antioxidants, including polyphenols, have been shown to have anti-microbial, anti-inflammatory, immunomodulatory, anti-aging, anti-asthmatic, and anti-cancer qualities in addition to reducing oxidative stress. Polyphenols also demonstrated hepatoprotective, cardioprotective, and neuroprotective properties [9]. The kind or source of polyphenols

and how they interact with the human microbiome determine these phytochemicals bioavailability. Numerous studies have demonstrated that polyphenols may indirectly enhance patients' health by modifying their gut microbiota, which may lessen systemic and intestinal inflammation by enhancing metabolic parameters [10]. One or more unpaired electrons in atomic or molecular orbitals are present in molecules that are free radicals [11]. Increased oxidative stress on cellular structure and alterations in molecular pathways that underlie the pathophysiology of several significant diseases, cancer, and the physiological aging process are caused by aberrant free radical generation, according to mounting data [12]. One of the main endocrine conditions that impacts the body's primary systems and might result in issues for several organs is diabetes [13]. Biguanides and sulphonylureas are examples of verbal hypoglycemic agents that are predictable medications used for treatment; nevertheless, a significant disadvantage of these medications is their unfavorable side effects [14]. Herbal remedies are gaining traction because they are safer, produce better results, and address health issues more successfully than commercially available medications [15].

METHODS

Collection of plant material

G. sylvestre fresh leaves were collected from the Bengaluru campus of GKVK. Haleshi C., Department of Botany, Davangere University, Davangere, authenticated this plant (Fig. 1) [16,17].

Preparation of plant extract

Methanol was extracted from the dry plant leaf powder using the Soxhlet extraction method. The methanol extract was then dried in a rotary evaporator to produce the appropriate extract, which was then frozen at -20°C until it was needed for further analysis [18].

Qualitative analysis of phytochemicals

The presence of the following phytoconstituents was determined by preliminary phytochemical analysis of freshly made extracts: alkaloids, cardiac glycosides, anthraquinones, tannins, phenols, terpenoids, steroids, saponins, and flavonoids. To determine the components, qualitative tests were used utilizing standard methods [19,20].

Quantitative analysis of phytochemicals

HPLC analysis

Before HPLC analysis, all methanolic extracts were produced, filtered through a 0.45 μm syringe membrane filter, and heated to 25°C in a column. The solvent was first filtered using a Millipore membrane filter with a 0.45 μm , 50 mm diameter, and then sonicated for 15 min in a Micro Clean 109 bath. Acetonitrile, water, and potassium dihydrogen phosphate were used as the mobile phase in the chromatography process. 1.6 mL/min flow rate for the duration of the analysis [21,22]. For a gradient elution with increasing polarity, a binary mobile phase including the solvents acetonitrile and water was utilized [23]. Acetonitrile was 6–20 v/v at 0–10 min, 80 v/v at 40 min, 90 v/v at 60 min, and 100 v/v at 65 min. This allowed for the best separation [24,25].

Antioxidant assay

1,1-diphenyl-2-picryl hydrazine (DPPH) free radical scavenging assay

Analysis of antioxidant activity was carried out by the DPPH method. Concentrations of ascorbic acid is 6.25, 12.5, 25, 50, 100. Concentrations of the test sample are 62.5, 125, 250, 500, 1000. The radical scavenging property of the material was quantitatively measured using, with slight changes to, the Mensor *et al.* (2001) method. 1 mL of DPPH (0.1 mM) was added to 1 mL of the sample at various concentrations in a methanolic solution, which was then left to react for 30 min at room temperature in the absence of light [26,27]. The absorbance at 517 nm was measured. Methanol was used as the blank, ascorbic acid was used as the reference standard, and DPPH in methanol without sample was used as the control. The reaction mixture exhibited greater free radical scavenging activity as evidenced by its reduced absorbance. Using the following formula, the ability to scavenge the DPPH radical was determined [28,29].

$$\% \text{ of radical scavenging activity} = \frac{[(\text{Abs Control} - \text{Abs Sample}) / \text{Abs control}] \times 100}$$

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of leaf extracts of *G. sylvestre*

G. sylvestre leaf extract phytochemical analysis using methanol solvent. *G. sylvestre* leaf extracts in methanol demonstrated the absence of alkaloids, anthraquinone, tannins, and flavonoids and the presence of phenols, terpenoids, steroids, saponins, and glycosides. Antioxidant and other biological activities are caused by the presence of the bioactive components (Fig. 2) [30].

The discussion of the findings from the qualitative analysis is provided below, the research work carried out on the medicinal plant. Several kinds of phytochemical ingredients were found in *G. sylvestre*, as indicated in Table 1. The phytochemical screening indicated the existence of a variety of active substances [31].

Quantitative phytochemical analysis of leaf extracts of *G. sylvestre*

In this study, the active chemical components were extracted from dried and leaf powder was used for HPLC analysis. *G. sylvestre* extract samples are shown in the HPLC chromatogram [3] (Fig. 3 and Table 2).

% of gymnemic acid (0.02%), deacyl gymnemic acid (2.50%), and gymnemagenin (0.61%) present in the *G. sylvestre* which were collected from the GKV campus, Bangalore.

In this study, the HPLC eluted *G. sylvestre* leaf sample total active ingredients were divided into various fractions according to the standard sample retention duration.



Fig. 1: *Gymnema sylvestre* plant

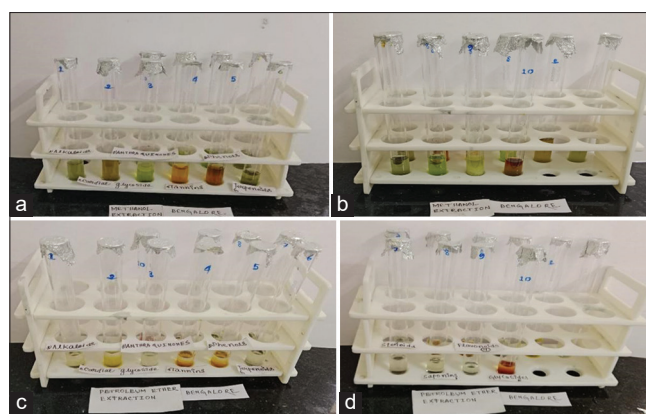


Fig. 2: Methanolic leaf extract (a) [Alkaloids-Terpenoids], (b) [Steroids-Glycoside], petroleum ether extraction, (c) [Alkaloids-Terpenoids], (d) [Steroids-Glycoside]

Table 1: Qualitative phytochemical analysis of leaf extracts of *Gymnema sylvestre*

S. No.	Phytochemicals	Methanol extract	Petroleum ether extract
1.	Alkaloids	–	–
2.	Cardiac glycoside	+	+
3.	Anthraquinone	–	–
4.	Tannins	–	–
5.	Phenols	+	+
6.	Terpenoids	+	–
7.	Steroids	+	–
8.	Saponins	+	–
9.	Flavonoids	–	+
10.	Glycoside	+	+

(+) denotes present and (–) denotes absent

Antioxidant activities

The most effective method of minimizing health risks and combating tissue damage and undesirable changes is through the antioxidant activity of medicinal plants [32]. One important indicator of the methanol plant extract's possible antioxidant activity could be its overall antioxidant activity. The DPPH radical assay, which primarily assesses proton radical scavenging ability, also demonstrated the plant extract's antioxidant activity. DPPH is among the chemicals that have a proton free radical with a distinctive absorption that dramatically drops when exposed to proton radical scavengers [33]. The plant extract in this investigation demonstrated concentration-dependent DPPH

Table 2: Quantitative phytochemical analysis of leaf extracts of *Gymnema sylvestre*

S. No.	Name of the ecotype	Place of collection	% of Deacyl gymnemic acid (w/w)	% of Gymnemagenin (w/w)	% of Gymnemic acid (w/w)
1.	Yelahanka (GKVK campus)	Bengaluru	2.50	0.61	0.02

Table 3: DPPH radical scavenging activity of test sample

S. No.	Sample	Conc. (µg/mL)	Abs at 517 nm	% of Inhibition
1	GSM	62.5	0.257±0.007	43.89±0.468
2		125	0.214±0.011	53.28±0.341
3		250	0.141±0.012	69.21±0.298
4		500	0.119±0.004	74.02±0.271
5		1000	0.078±0.010	82.97 ±0.288

Table 4: Free radical scavenging activity of ascorbic acid

S. No.	Sample	Conc. (µg/ml)	Abs at 517 nm	% of Inhibition
1	Ascorbic	62.5	0.730±0.019	39.17±0.352
2	acid	12.5	0.600±0.015	50.00±0.188
3		25	0.482±0.012	59.83±0.291
4		50	0.321±0.032	73.25±0.395
5		100	0.131±0.015	89.08±0.447

radical scavenging, which could be related to its capacity to donate hydrogen. The antioxidant activities of *G. sylvestre* methanolic extract were determined using the DPPH assay. The antioxidant activity data were compared with the positive control (ascorbic acid) and expressed as a percentage of inhibition [27] (Tables 3 and 4).

Due to safety concerns, synthetic antioxidants have been substituted by plants as a possible source of fresh antioxidants, according to preventative medicine [34]. Certain photochemicals have been shown to be responsible for particular activities. For example, steroids have the ability to induce inflammation, alkaloids have antibacterial, analgesic, and other antispasmodic effects, and flavonoids have the potential to be antioxidants [35-37]. Based on the findings of this research, the DPPH experiment revealed that *G. sylvestre* methanolic extract exhibited significant dose-dependent antioxidant activity [38,39], with an 50% inhibitory concentration (IC_{50}) of 89.42 µg/mL. Antioxidants are compounds that protect cells against the damaging effects of ROS, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals, and peroxynitrite. An imbalance between antioxidants and ROS results in oxidative stress, leading to cellular damage [40]. Cancer, aging, atherosclerosis, inflammation, ischemia injury, and neurodegenerative illnesses have all been connected to oxidative stress. The DPPH radical scavenging activity of *Gymnema sylvestre* methanolic extract (GSM) showed a clear dose-dependent increase in antioxidant activity in Table 3. As the concentration increased from 62.5 µg/ml to 1000 µg/ml, the % inhibition rose significantly from 43.89±0.46 to 82.97±0.28. This suggests that the extract has strong free radical scavenging potential, which may be due to the presence of phenolic compounds and gymnemic acids. The decreasing absorbance values at 517 nm also indicate increasing radical scavenging efficiency. These findings support the traditional use of *Gymnema sylvestre* in herbal medicine for its antioxidant properties [41,42].

The DPPH free radical scavenging activity of ascorbic acid, as presented in Table 4, demonstrates a clear concentration of 62.5 µg/ml, the percentage of inhibition was 39.17 ±, which progressively increased with concentration. A moderate activity was observed at 25 µg/ml (59.83±), while the highest inhibition of 89.08± was recorded at 100 µg/ml. The decreasing absorbance values at 517 nm with increasing concentrations indicate effective neutralization of DPPH radicals by ascorbic acid. These results confirm the potent antioxidant nature of

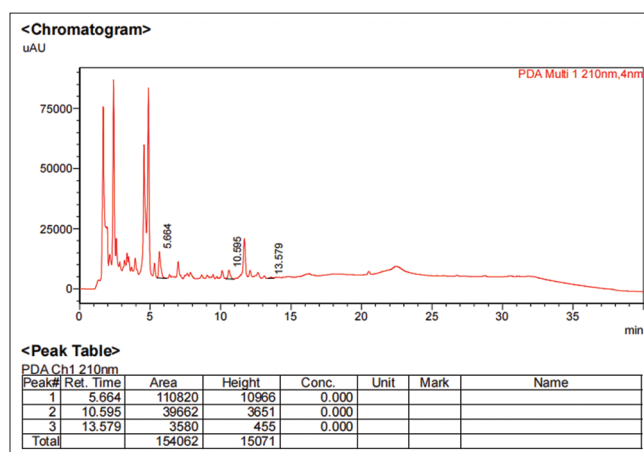
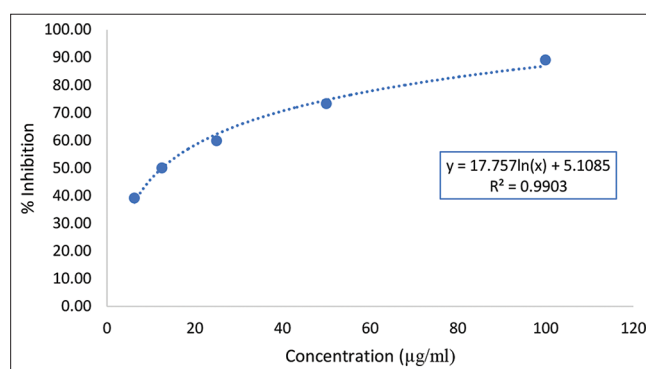
Fig. 3: High-performance liquid chromatography chromatogram of leaf extracts of *Gymnema sylvestre*

Fig. 4: Graph of standard ascorbic acid

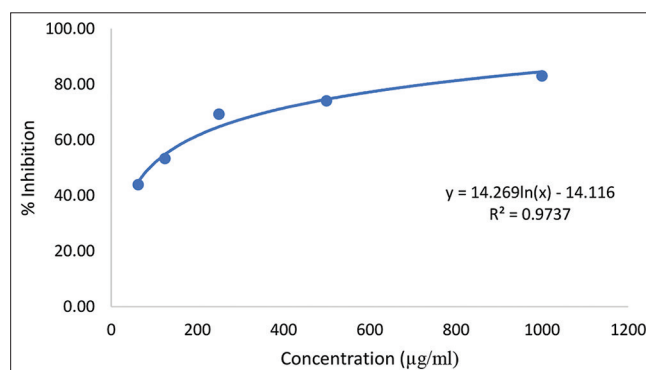


Fig. 5: Graph of test-sample

ascorbic acid, consistent with previous reports highlighting its role as a standard antioxidant in free radical scavenging assays. The data also support the use of ascorbic acid as a positive control in comparative antioxidant studies of plant extracts.

The coefficient of determination $R^2 = 0.9903$ indicates a very strong correlation between concentration and inhibition (Figure 4). Standard

ascorbic acid exhibits potent antioxidant activity, with % of inhibition increasing logarithmically with concentration. The high R² value validates the reliability of the data.

Figure 5 graph shows a dose-dependent increase in DPPH free radical scavenging activity with increasing concentrations of the test sample (in µg/ml). At lower concentrations, the % of inhibition was around 40-50%, and as the concentration increased, the inhibition rose gradually, reaching above 80% at the highest concentration tested. The R² value of 0.9737 indicates a strong positive correlation between the concentration of the sample and its antioxidant activity. The strong correlation and high percentage of inhibition indicate that the sample may contain potent free radical scavenging compounds.

CONCLUSION

We can conclude that when compared to ascorbic acid, the methanolic extract of *G. sylvestre* exhibits significant dose-dependent antioxidant properties. Alkaloids, steroids, proteins, terpenoids, glycosides, and other phytochemicals may be responsible for the plant extract's strong antioxidant action. Additional research is necessary to replace plant extract as a natural medication for the treatment of diseases brought on by free radicals. In the plant materials examined in this study, bioactive phytocompounds were found. The phytocompounds found in medicinal plants are an essential source of molecules that may improve overall health. Plants used for medicinal purposes are an important source of phytocompounds that may improve overall health. Research such as this one is continuing to characterize the bioactivities of herbal items and expand their use in medicine.

AUTHOR'S CONTRIBUTION

Suchitra Boxi, Haleshi C, for collecting the sample, data collection, analyzing the data, and writing the manuscript. Prakash N.S for providing HPLC Data with the respective standard.

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CONFLICT OF INTEREST

I declare that I have no conflict of interest.

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