Research Article

Efficacy of *Gymnema Sylvestre* as a Potent Antioxidant: An *In Vitro* Study

Keerthika R and Sandhya Raghu*

Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Corresponding author: Sandhya Raghu, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India; Email: sandhya@gmail.com

Abstract

Background: Antioxidants are the substances which act as radical scavengers and help in conversion of reactive oxygen species, thus preventing the cellular damage. In recent times the synthetic antioxidants are substituted by natural antioxidant, which are primarily derived from plant sources. It has been proven that these antioxidant properties of the medicinal plants are mainly due to its phytochemical constituents. *Gymnema sylvestre* is one such traditional ayurvedic herb used primarily for the treatment of diabetes and other ailments such as arthritis, anemia, diuretics, etc. **Aim:** The aim of this study is to analyse the antioxidant potential of ethanolic extract of *Gymnema sylvestre* in comparison with the ascorbic acid. **Materials & Methods:** The ethanolic extract of *Gymnema sylvestre* was prepared and the antioxidant potential of the extract at different concentrations (25,50,100,200 µg/ml) was tested using DPPH assay and ABTS radical scavenging activity and reducing power assays are designated by their IC_{50} (concentration required to attain 50% radical-scavenging effect) and compared with that of the standards(Ascorbic acid). **Results:** It is shown that there is concentration dependent antioxidant activity of ethanolic extract of *Gymnema sylvestre* with IC50 of *Gymnema sylvestre* found to be 74.8 µg/ml and 83.8 µg/ml by ABTS andDPPH assay respectively. **Conclusion:** Within the limitations of this study it can be concluded that ethanolic extract of *Gymnema sylvestre* has considerable dose dependent antioxidant property when compared withascorbic acid. However, further preclinical and *ex vivo* studies have to be carried to prove its use in clinical trials.

Keywords: Antioxidant; *Gymnemasylvestre*; DPPH assay; ABTS radical scavenging activity

Introduction

Antioxidants are the fundamental elements in the human biological system that help to maintain the balance between the oxidation and antioxidation and also help to scavenge the free radicals like reactive oxygen species, which damages the DNA and lead to the oxidation of lipids and proteins in cells. [1]

The exogenous sources of antioxidants are mainly from medicinal plants such as fruits, leaves, stems, barks, roots, etc. [2,3]

Gymnema sylvestre is a perennial woody vine native to tropical Asia, China, Africa and Australia. [4,5]

It has been traditionally used in ayurvedic medicine for treating various illnesses such as diabetes, [6] arthritis, diuretics, anemia, hypercholesterolemia etc. [7,8] It also shows antibacterial, anti-inflammatory and anti- carcinogenic properties. [9]

With the increasing trend of replacingthe synthetic antioxidants, mainly for the safety concern with the available natural antioxidants has paved its way for this study.

The aim of this study was to analyze the antioxidant potential of ethanolic extract of *Gymnema sylvestre*.

Materials and Methods

Extract preparation

For preparing extract, 500 gm. of the powdered Gymnema sylvestre sample soaked with 70% ethanol (in ratio ethanol:plant (6:1)) for 72 hrs and filtered using Whatman No. 1 paper.

How to cite this article: Keerthika R, et al. Efficacy of *Gymnema Sylvestre* as a Potent Antioxidant: An *In Vitro* Study. Ann Med Health Sci Res.2021;11:232-236

The filtrate was placed into the thimble of the Soxhlet extraction apparatus chamber. The sample was extracted for 12 hours at 4 cycles per hour.

After extraction, the solvent was removed by the means of a vacuum evaporator, yielding the extracted compound. The crude extracts were weighed and stored at 0°C-4°C before analysis.

Chemicals and reagents

1,1-Diphenyl-2-Picrylhydrazyl (DPPH), ascorbic acid, ABTS, potassium persulphate and all other chemicals including solvents were of analytical grade procured from SD fine chemicals. [10]

The antioxidant potential of the extract was determined by DPPH and ABTS radical scavenging activity and reducing power assays are designated by their IC50 (concentration required to attain 50% radical-scavenging effect) and compared with that of the standards (Ascorbic acid). [11]

DPPH assay

For DPPH scavenging activity ethanol solution of plant extract at different concentrations (25–20 µg/ml) was mixed with 0.8 ml of 100 mm trisHCl buffer adjusted to pH 7.4. DPPH (500 mm in 1.0 ml ethanol) solution was added to the above mixture to the test tubes.

The mixture was shaken vigorously and incubated for 30 min at room temperature. Absorbance of the resulting solution was measured at 517 nm UV-Visible Spectrophotometer (Labomed).

All the assays were carried out in triplicates. The ascorbic acid was used as a standard antioxidant in this method. Percentage of DPPH scavenging activity was determined. [12]

ABTS assay

ABTS radical cation was produced by the reaction of a 7 mmol/L ABTS solution with 2.45 mmol/L potassium persulphate. The mixture was stored in the dark at room temperature for 12 hrs. before use. The ABTS+ solution was diluted with ethanol and added to the mixture to read an absorbance at 734 nm. After addition of 25 µl of sample or standard to 2 ml of diluted ABTS+ solution, absorbance at 734 nm was read after 6 min. A standard curve was prepared by measuring the reduction in absorbance of ABTS•+ solution at different concentrations of extract. Appropriate blank measurements were carried out and the values recorded. Ascorbic acid was used as positive control. [13]

Statistical analysis

Results will be expressed as mean ± S.E.M. Statistical significance was determined by one-way Analysis Of Variance (ANOVA), followed by a Dunnett's multiple-comparison test with 95% confidence intervals. P values less than 0.05 were considered significant.

Results

The antioxidant activities of *Gymnema sylvestre* ethanolic extract were determined by using DPPH assay and ABTS assay. The results of antioxidant activity were expressed in terms of percentage of inhibition (%) and compared with the positive control (ascorbic acid) (Table 1 and Table 2).

From the Figure 1 and Figure 2, it is shown that there is concentration dependent antioxidant activity of ethanolic extract of *Gymnema sylvestre*. IC50 of *Gymnema sylvestre* was found to be 74.8 µg/ml and 83.8 µg/ml by ABTS and DPPH assay respectively.

Table 1: ABTS radical scavenging activity.

Sample	Conc. (µg/ml)	Abs at 734 nm	% of Inhibition
GSE	25	0.365 ± 0.02	12.5 ± 1.1***b
	50	0.276 ± 0.01	33.4 ± 1.9***b
	100	0.201 ± 0.09	51.5 ± 2.4***b
	200	0.156 ± 0.13	62.4 ± 6.4***a
Ascorbic acid	25	0.263 ± 0.09	41.4 ± 2.3***
	50	0.157 ± 0.04	62.1 ± 3.7***
	100	0.119 ± 0.08	71.3 ± 4.2***
	200	0.063 ± 0.13	84.9 ± 7.3***
Negative control		0.415 ± 0.03	0.0 ± 0.0

Results are expressed as Mean ± SEM. ***p<0.001 statistically significant as compared with Negative control; bp<0.01;ap<0.05 statistically significant as compared with ascorbic acid.

GSE: Gymnema sylvestre ethanolic extract.
IC50 of GSE:74.8 µg/ml.

Table 2: DPPH radical scavenging activity.

Sample	Conc. (µg/ml)	Abs at 517 nm	% of Inhibition
GSE	25	0.409 ± 0.23	16.3 ± 1.4***b
	50	0.343 ± 0.17	29.8 ± 2.5*** b
	100	0.201 ± 0.16	58.8 ± 2.8***b
	200	0.126 ± 0.189	74.2 ± 4.3***a
Ascorbic acid	25	0.379 ± 0.21	22.4 ± 1.8***
	50	0.238 ± 0.14	51.3 ± 2.6***
	100	0.169 ± 0.39	65.4 ± 3.2***
	200	0.086 ± 0.05	74.2 ± 3.7***
Negative control		0.489 ± 0.23	0.0 ± 0.0

Results are expressed as Mean ± SEM. ***p<0.001 statistically significant as compared with Negative control. bp<0.01; ap<0.05 statistically significant as compared with ascorbic acid. GSE: *Gymnema sylvestre* ethanolic extract. IC50 of *Gymnema sylvestre* extract: 83.8 µg/ml.

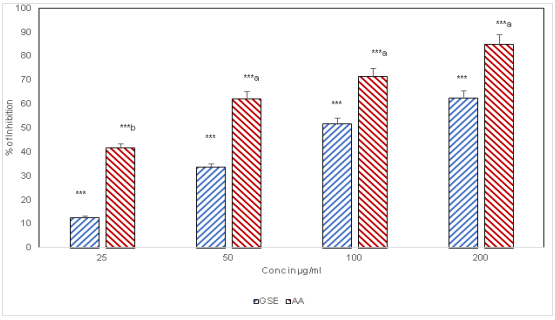

Figure 1: Graph depicts the percentage of ABTS free radical inhibition activity.
This graph depicts the percentage of ABTS free radical inhibition activity. Results are expressed as Mean ± SEM. ***p<0.001 statistically significant as compared with Negative control; bp<0.01; ap<0.05 statistically significant as compared with ascorbic acid. GSE: *Gymnema sylvestre* ethanol extract; AA: Ascorbic Acid.

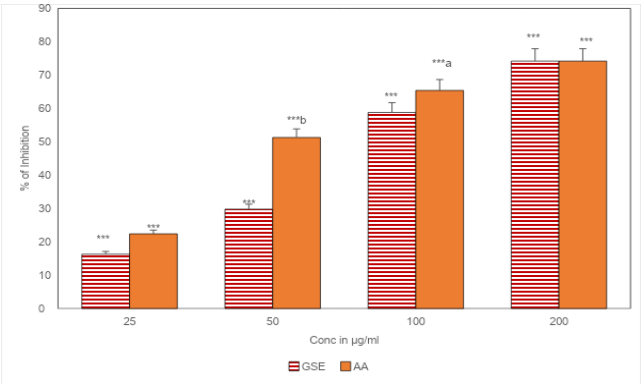

Figure 2: Graph depicts the percentage of DPPH radical scavenging action.
This graph depicts the percentage of DPPH radical scavenging action. Results are expressed as Mean ± SEM. bp<0.01; ap<0.05 statistically significant as compared with ascorbic acid. IC50 of *Gymnema sylvestre* extract: 83.8 µg/ml.

Discussion

According to preventive medicine, plants are considered as a potential source of new antioxidants which have replaced the synthetic antioxidants due to safety concerns. [14]

Gymnema sylvestre which is primarily used for its antidiabetic property, possess various phytochemicals such as flavonoids, tannins, alkaloids, phenols, ascorbic acid, folic acid etc. [15–17]

Research has proved that specific photo chemicals are responsible for the specific activity such as flavonoids has antioxidant potential while alkaloids have antimicrobial, analgesic, and other antispasmodic actions and steroids have inflammatory potency. [18]

A study by Rahman et al., has shown that the ethanolic extract of *Gymnema sylvestre* has antioxidant potential and also its potential is better than that of *A. bilimbi* and *C. frutescens*. [19]

The antioxidant activity of *Gymnema sylvestre* was also investigated by Rupanar and Ohmori against butylated hydroxyl toluene, proving it possesses better DPPH scavenging activity and also to reduce LDL oxidation. [20]

In another study, showed the scavenging activity of hydroxyl free radical and antioxidative potential of this plant. [21]

Studies have proven that the extract have antioxidant potential in conditions high fat diet, hydrogen peroxide, nitric oxide and superoxide radical induced oxidative stress in rats. [22–25]

Rachh et al. has shown that the methanolic extract of *Gymnema sylvestre* having significant radical scavenging activity against ferric, super oxide, hydrogen peroxide.

Previous studies has shown the efficacy of methanolic extract of *Gymnema sylvestre* as potent antioxidant but there are limited studies using its ethanolicextract. [26]

According to the results of this study, the ethanolic extract of *Gymnema sylvestre* showed considerable dose dependent antioxidant activity with IC50 of *Gymnema sylvestre* was found to be 74.8 µg/ml and 83.8 µg/ml by ABTS and DPPH assay respectively which was in accordance with the study by Behera et al. [26]

This result suggests that ethanolic extract of *Gymnema sylvestre* has dose dependent antioxidant activity, recommending its use as alternative to synthetic antioxidants.

Conclusion

Within the limitations of this study it can be concluded that ethanolic extract of *Gymnema sylvestre* has considerable dose dependent antioxidant when compared with ascorbic acid.

However, further preclinical and *ex vivo* studies have to be carried to prove its use in clinical trials.

References

1. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, et al. Natural antioxidants in foods and medicinal plants: Extraction, Assessment and resources. Int J Mol Sci. 2017;18:96.
2. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74:2157-84.
3. Bin S, Yizhong ZC, Mei S, Harold C. J Agric Food Chem. 2005;53:7749-59.
4. Laha S, Paul S. *Gymnema sylvestre* (Gurmar): A potent herb with anti-diabetic and antioxidant potential. Pharmacog J. 2019;11: 201-206.
5. Dutta S, Das S. A study of the anti-inflammatory effect of the leaves of Psidium guajava Linn. on experimental animal models. Pharmacognosy Res. 2010;2:313–7.
6. Pragya T, Mishra BN, Neelam SS. Phytochemical and pharmacological properties of *Gymnema sylvestre*: An important medicinal plant. Biomed Res Int.2014830285.
7. Singh DK, Kumar N, Sachan A, Lakhani P, Tutu S, Nath R, et al. Hypolipidaemic effects of *Gymnema sylvestre* on high fat diet induced Dyslipidaemia in Wistar Rats. JCDR. 2017;11:FF01-FF05.
8. Farzana K, Moklesur RS, Long CM, Isa Naina M, Chao Z, Bassem YS, et al. Comprehensive review on phytochemicals, pharmacological and clinical potentials of *Gymnema sylvestre*. Front Pharmacol. 2019;10:1223.
9. Ramalingam R, Dhand C, Leung CM, Ong ST, Annamalai SK, Kamruddin M, et al. Poly-ε-Caprolactone/Gelatin hybrid electrospun composite nanofibrous mats containing ultrasoundassisted herbal extract: Antimicrobial and cell proliferation study. Nanomaterials (Basel). 2019;9:462.
10. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal. 2014;22: 296–302.
11. Badami S, Channabasavaraj KP. *In Vitro* antioxidant activity of thirteen medicinal plants of India's western ghats. Pharm Bio. 2007; 45:392-396.
12. Meena H, Pandey HK, Pandey P, Arya MC, Ahmed Z. Evaluation of antioxidant activity of two important memory enhancing medicinal plants *Baccopa monnieri* and *Centella asiatica*. Indian J Pharmacol. 2012;44:114-7.
13. Agnieszka TC, Mateusz G, Elżbieta R, Agnieszka Ki, Alicja ZK, Anna SŁ. Study of antioxidant activity of some medicinal plants having high content of caffeic acid derivatives. Antioxidants (Basel). 2020;9:412.
14. Wilfred MO, Donald SG, Roland NN. Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. BMC Complement Altern Med. 2012;12:43.
15. Myung K, Min SL, Mi KC, Kwan SM, Takayuki S. Hypoglycemic activity of *Gymnema sylvestre* extracts on oxidative stress and antioxidant status in diabetic rats. J Agric Food Chem. 2012;60:2517-24.
16. Rose RC, Bode AM. Biology of free radical scavengers: An evaluation of ascorbate. FASEB J.1993;7:1135-1142.
17. Rachh PR, Rachh MR, Ghadiya NR, Modi DC, Modi KP, Patel NM, Rupareliya MT. Antihyperlipidemic activity of *Gymenma sylvestre* R. Br. Leaf extract on rats fed with high cholesterol diet. Int J Pharmacol. 2010;6:138-141.
18. Hassan A, Akmal Z, Khan N. The phytochemical screening and antioxidants potential of *Schoenoplectus triqueter* L. Palla. J Chem. 2020;1.
19. Rahman MM, Habib MR, Hasan MA, Amin MA, Saha A, Mannan A. Comparative assessment on *in vitro* antioxidant activities of ethanol extracts of *Averrhoa bilimbi*, *Gymnema sylvestre* and *Capsicum frutescens*. Pharmacognosy Res. 2014;6:36-41.
20. Ohmori R, Iwamoto T, Tago M, Takeo T, Unno T, Itakura H, et al. Antioxidant activity of various teas against free radicals and LDL oxidation. Lipids. 2005;40:849-53.
21. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced Hyperinflammation magnify the severity of coronavirus disease (CoViD-19) leading to acute respiratory distress syndrome? Front Immunol. 2020;27;11:1206.
22. Karthikeyan P, Chakrapani LN, Mohan T, Tamilarasan B, Kannan P, Periandavan K. Gymnemic acid, a potent antidiabetic agent protects skeletal muscle from hyperglycemia mediated oxidative stress and apoptotic events in High fat and high fructose diet fed adult rats. Int J Res Pharm Sci. 2020;11:1526-1538.
23. Vadivel JK, Govindarajan M, Somasundaram E, Muthukrishnan A. mast cell expression in oral lichen planus: A systematic review. J Investig Clin Dent. 2019;10:e12457.
24. Ponnanikajamideen M, Rajeshkumar S, Vanaja M, Annadurai G. *In vivo* type 2 diabetes and wound-healing effects of antioxidant gold nanoparticles synthesized using the insulin plant *Chamaecostus cuspidatus* in albino rats. Can J Diabetes. 2019;43:82–9.e6.
25. Arun LB, Arunachalam AM, Arunachalam KD, Annamalai SK, Kumar KA. *In vivo* anti-ulcer, anti-stress, anti-allergic, and

functional properties of Gymnemic Acid Isolated from *Gymnema sylvestre* R Br. BMC Complement. Altern Med. 2014;14:70.

26. Behera SK. Phytochemical analysis and antioxidant activities of *Gymnema sylvestre* R. Br. Leaf Extracts. Free Radicals and Antioxidants. 2019;9:12-15.