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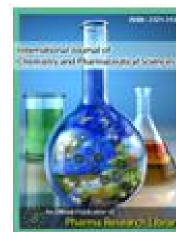

Research Article **Open Access**

Phytochemical Screening and Evaluation of the Antioxidant and Anti-Inflammatory Potentials of *Gymnema sylvestre* Leaves

Sangeetha Sathyanarayan, Dr. P.T. Srinivasan, S. Prasanth

PG and Research Department of Biochemistry, D.G. Vaishnav College, Arumbakkam, Chennai 600106.

A B S T R A C T

The aim of the present study is to look for antioxidant and anti inflammatory activities in the different extracts of *Gymnema sylvestre* leaves. The preliminary screening of the leaf extracts reveal the presence of phytochemicals like alkaloids, flavonoids, saponins, steroids, glycosides, tannins and phenol in the different extracts of the seven extracts studied, ethanol, 40% ethanol and acetone extracts were found to be richest in their phytochemical composition. Antioxidant components like phenols, flavonoids and flavonols are rich in Gymnema extracted with the hydroalcohol (40% ethanol) fraction showing maximum. The 40% ethanolic extract of Gymnema sylvestre exhibited potent antioxidant activity inhibiting DPPH radicals. It also showed the highest total antioxidant capacity. The anti inflammatory potential was evaluated in the aqueous and ethanolic extracts and was found to be appreciable in the ethanolic extracts. The findings confirm the potential benefits of the plant as described in traditional medicine.

Keywords: Gymnema sylvestre, phytochemicals, antioxidant, anti inflammatory.

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***Corresponding Author**
Dr. P.T. Srinivasan
Department of Biochemistry,
D.G. Vaishnav College, Chennai
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1. Introduction

India is a country of rich and diverse heritage. The use plants for healing as well as for curing human disease has been practiced here since time immemorial. In the recent past, the medicinal properties of plants have been attributed

to the presence of their phytochemical constituents. Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, roots, flower, fruits, seeds, etc. i.e. any part of the body may contain active component [1]. These metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities. Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global health problem [2]. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances [1]. *Gymnema sylvestre* R. Br. is a valuable herb belonging to the family Asclepiadaceae and widely distributed in India, Malaysia, Srilanka, Australia, Indonesia, Japan, Vietnam, tropical Africa and the southwestern region of the People's Republic of China. The plant is commonly known as Periploca of the woods (English); Gurmar (Hindi); Meshashringi, madhunashini (Sanskrit); Kavali, kalikardori (Marathi); Dhuleti, mardashingi (Gujrathi); Adigam, cherukurinja (Tamil) [3].

2. Experimental

Plant Material

The plant *Gymnema sylvestre* was collected from the neighbourhood. The plants were identified and authenticated Dr. Prof. P. Jayaraman, Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram.

Extract Preparation:

Dried and powdered plant material was extracted using water, methanol, ethanol, aqueous ethanol (40%), benzene, chloroform, and acetone. The crude extract was filtered and evaporated to complete dryness at room temperature. The percentage yield of extract was calculated.

Phytochemical Screening:

Preliminary phytochemical examinations were carried out for all the extracts as per the standard methods. [4-6].

Quantitative Analysis of Antioxidant Components

Total Phenolics determination

The total phenolic content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, different concentrations of the extracts were mixed with 0.4ml FCR (diluted 1:10v/v). After 5 minutes 4ml of sodium carbonate solution was added. The final volume in the tubes were made up to 10ml with distilled water and allowed to stand for 90 minutes at room temperature. Absorbance of sample was measured against the blank at 670 nm using a colorimeter (ELICO CL63 PHOTOMETER). A calibration curve was constructed using gallic acid solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of gallic acid per gram of dry weight.

Total Flavonoid and Flavonol determination

Total flavonoid content was determined by Aluminium chloride method using quecertin as a standard. 1ml of test sample and 4ml of water were added to a volumetric flask (10ml volume). 5 minutes after adding 0.3 ml of 5% sodium nitrite, 0.3ml of 10% Aluminium chloride was added. After 6 minutes incubation at room temperature, 2ml of 1M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made upto 10ml with distilled water. The absorbance of the reaction mixture was measured at 510nm against a blank colorimetrically (ELICO CL63 Photometer). Results were expressed as quecertin equivalents (mg quecertin /g dried extract). Total flavonols were estimated as quecertin equivalents, expressed as mg of quecertin per gram of dry extract by the method of Miliauskas et al. The calibration curve was prepared by mixing Quecertin solution with 2 ml $AlCl_3$ (20 gm/l) and 6ml sodium acetate (50 gm/l) The absorption at 440 nm was read after 2.5 h at 20°C. The same procedure was carried out with 2ml of plant extract (10 gm/l) instead of quecertin solution All determinations were carried out in triplicates and the mean values were used [7].

Assessment of *In-vitro* Antioxidant Activity

Total Antioxidant Capacity

For total antioxidant capacity assay, 0.3 ml of the gymnema extract (10 mg/ml) dissolved in water and mixed with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate) in Eppendorf tube. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After 90 min, the mixture was cooled to room temperature. The absorbance was measured at 695 nm against reagent blank. Methanol (0.3 ml) in the place of extract is used as the blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

DPPH Radical Scavenging Activity

Plant extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80 and 100 µg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition activity was calculated from $[(A_0–A_1)/A_0]\times100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations [8].

Assessment of *In vitro* Anti- InflammatoryActivity

Inhibition of albumin denaturation [9]

Method of Mizushima et al was followed with minor modifications. The reaction mixture consisting of test extract at different concentrations and 1% aqueous solution of bovine serum albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37°C for 20 minutes and then heated at 57°C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

Percentage inhibition =$(A_{bscontrol}–A_{bssample})$X100/ Abscontrol

Heat induced hemolysis [10]

The reaction mixture (2 ml) consisted of 1 ml of test drug solution and 1mL of 10%RBC. Instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates.

3. Results and Discussion

In the present investigation, the primary and secondary metabolites in the leaves of *Gymnema sylvestre* were qualitatively and quantitatively analysed. The organoleptic evaluation of the leaf powder is depicted below.

Table 1: Organoleptic characters of the leaf powder

Colour	Green - Greenish yellow
Odour	Characteristic
Taste	Astringent

Table 2: Physicochemical evaluation of the leaf powder

Moisture content	9%
Ash content	89%

Insufficient drying favours spoilage by molds and bacteria and make possible the enzymatic destruction of active principles. Not only the ultimate dryness of the drug is important, equally important is the rate at which moisture is removed and the conditions under which it is removed. Thus, the determination of moisture content also provide the method of preparation of the drug [11,12].

The results of the physicochemical contents signify that the quality and purity of the raw material was satisfactory .Dried and powdered leaves were extracted using polar and non polar solvents & screened for their phytochemical constituents. The results are presented in Table 3,4&5. Table 3 reveals the presence of a number of plant constituents such as tannins, saponins, flavonoids, alkaloids, steroids, phenols, diterpenes, in the fresh extracts of *Gymnema sylvestre* .

Table 3: Preliminary phytochemical Screening of crude extract

Fresh extract	Aqueous	Methanol	Ethanol	40% Ethanol	Acetone	Chloroform	Benzene
Tannins	++	+++	+	++	++	+	++
Carbohydrate	-	-	-	-	-	-	-
Saponins	++	+	+	++	+	+	+
Flavanoids	++	++	+	+	+++	+	+
Alkaloids	++	++	+	++	+++	+++	+
Steroids	+	+	-	-	+	+	++
Phenols	+	++	+	+	++	+	++
Glycosides	+	++	+	+	+++	+	++
Proteins/Aminoacids	++	++	+	+	+	++	+
Diterpenes	+	+	++	++	+++	-	+
Resins	++	+++	+++	+++	++	-	+++

The extracts were then stored in light and dark (away from light) conditions for a period of 30 days. The phytochemical screening was repeated to look for changes in its composition. The results are tabulated below.

Table 4: Phytochemical screening of extract stored in normal condition

Light extract	Aqueous	Methanol	Ethanol	40% Ethanol	Acetone	Chloroform	Benzene
Tannins	+	+	++	+ +	+	+	+
Carbohydrate	+	+	+	+	+-	+	+
Saponins	++	+	++	++	+	+	+
Flavanoids	+	++	++	+++	++	+	+++
Alkaloids	++	++	++	++	+++	+++	+
Steroids	+	+	+	+	+	+	+
Phenols	+	++	++	+	+	+	++
Glycosides	-	+	+	+	+++	+	+
Proteins/Aminoacids	+	+	+	+	+	+	+
Diterpenes	+	++	++	+	+	-	++
Resins	++	+++	++	++	+	-	++

Table 5: Phytochemical screening of extract stored away from light

Dark extract	Aqueous	Methanol	Ethanol	40% Ethanol	Acetone	Chloroform	Benzene
Tannins	++	+	++	+	+	+	+++
Carbohydrate	-	-	-	-	-	-	-
Saponins	+++	+	+	+	+	+	++
Flavanoids	+	++	+++	++	++	+	++
Alkaloids	+++	+	+	+	++	+++	+
Steroids	+	+	+	+	+	+	+++
Phenols	+	++	+	+	+	+	++
Glycosides	+	++	+	+	+++	+	+
Proteins/Aminoacids	++	+	+	++	+	+	+
Diterpenes	+	++	++	+	++	-	+
Resins	++	++	++	++	+	-	+

The extracts did not show appreciable variation indicating stability of the constituents for a good shelf life. The total phenolic content in all the solvent extracts were estimated by folin ciocalteu's method using gallic acid as standard , while total flavonoid and flavonol contents were measured with the aluminium chloride using quecertin as standard and the results are tabulated below.

Table 6: Antioxidant components in leaf extracts

Concentration of the extract (10 mgs /mL)	Phenol content (mgs of gallic acid eq/200mg of dried exracted material)	Flavanoid content(mgs of quecertin eq/200 mgs of dried extracted material)	Flavanol content(mgs of quecertin eq/200 mgs of dried extracted material)
Aqueous	41.79	75	100
Methanol	41.04	50	165
Ethanol	45.5	87	100
Hydro ethanol	45.5	62.5	150
Benzene	41.79	123.5	50
Acetone	45.5	87.5	50
Chloroform	44.78	37.5	100

The results indicate that the aqueous, methanol, ethanol and hydroethanol extracts show an overall higher content of the above components and hence were used for further analysis.

Table 7: Total antioxidant capacity

Leaf extract concentration (10 mg/ml)	mgs/gm expressed as ascorbic acid
Methanol	56.66
Ethanol	51.3
Hydroalcohol	66.4

The total anti oxidant capacity in the gymnema extract was determined by the formation of the phosphomolybdenum complex.

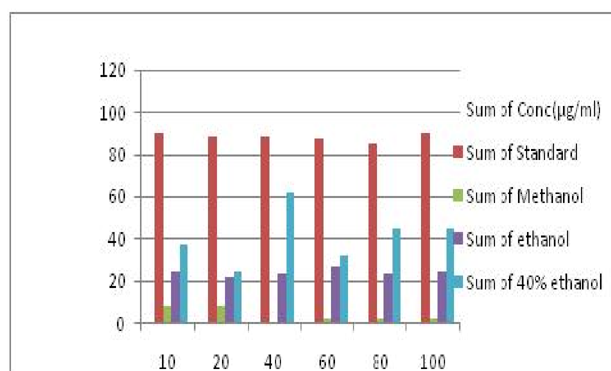

Figure 1: DPPH radical scavenging activity of gymnema extract

The gymnema extract was able to reduce the stable radical DPPH to the yellow coloured diphenyl picrylhydrazine. From the figure it is evident that the free radical scavenging potential of the hydro ethanol extract is the maximum at a concentration of 40 µg/ml and decreases with further increase in its concentration.

Table 8: Percentage inhibition demonstrated in the different extracts

Groups	Heat induced hemolysis	Albumin denaturation
Control	_	_
Aqueous extract	41.1	44
Ethanolic exract	62.3	66
Standard (Dicyclofenac)	63.12	82.6

The extracts inhibited the heat induced hemolysis of RBCs to varying degree. The maximum inhibition of 62.3 % was recorded in the ethanolic extracts followed by aqueous extracts showing 41.1% inhibition. The results were compared with standard Diclofenac sodium which showed the maximum inhibition of 63.12% at 100mg/ml. The results are reported in table 8. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract to prevent denaturation was studied. The ethanolic extracts were found to be effective in inhibiting heat induced albumin denaturation in comparison to aqueous extracts at a dose of 100mg/ml. Maximum inhibition 66% was observed from ethanolic extracts followed by aqueous extracts (44%).The results were compared with standard Diclofenac sodium which showed 82.6% inhibition in albumin denaturation. The results are reported in table 8 .

4. Conclusion

The preliminary screening of the leaf extracts reveal the presence of phytochemicals like alkaloids, flavonoids, saponins, steroids, glycosides, tannins and phenol in the different extracts. The ethanol, 40% ethanol and acetone extracts were found to be richest in their phytochemical composition. Antioxidant components like phenols, flavonoids and flavonols are richest in Gymnema hydroalcohol (40% ethanol). The present study revealed that the 40% ethanolic extract of *Gymnema sylvestre* exhibited potent antioxidant activity inhibiting DPPH radicals. It also showed the highest total antioxidant capacity. The anti inflammatory potential was evaluated in the aqueous and ethanolic extracts and was found to be appreciable in the ethanolic extracts. Further studies to evaluate the in vivo antioxidant and anti inflammatory potentials of gymnema extract are needed in order to make this valuable herbal formulation available for human consumption.

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