



ISSN 2320-3862

JMPS 2014; 2(4): 19-23

© 2014 JMPS

Received: 24-05-2014

Accepted: 04-06-2014

Kalyani Singh

Regional Plant Resource Centre
(RPRC), Nayapalli, Bhubaneswar-
15, Odisha, India

Bandita Deo

Regional Plant Resource Centre
(RPRC), Nayapalli, Bhubaneswar-
15, Odisha, India

Phytochemical evaluation and *in vitro* antioxidant activity of *Gymnema sylvestre* R.Br.

Kalyani Singh, Bandita Deo

ABSTRACT

The present study was carried out to evaluate the *in vitro* antioxidant activity and preliminary phytochemical analysis of ethanolic, methanolic and aqueous leaf extracts of *Gymnema sylvestre* R.Br. The *in vitro* antioxidant activity was evaluated by DPPH radical scavenging activity method. The *Gymnema sylvestre* R.Br. ethanolic extract showed antioxidant activity by inhibiting DPPH. Significant antioxidant activity of ethanolic leaf extract of *Gymnema Sylvestre* R.Br. might be due to the presence of tannins, saponins, phenols, flavonoids and alkaloids found in the preliminary phytochemical analysis.

Keywords: *Gymnema sylvestre*, Antioxidant activity, Phytochemicals, DPPH.

1. Introduction

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, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components [1].

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 [2]. 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 antioxidant [3]. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [4].

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second [5]. Antioxidants are added as redox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical induced decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule [6].

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 [7]. *Gymnema sylvestre* called as Gurmar in Hindi and Periploca of the Woods in English is a highly effective anti-diabetic medicinal herb. Leaves contain lupeol, β -amyrin, stigmaterol, pentriacontane, hentricontane, α and β chlorophyll, resin, tartaric acid, gymnemic acid (anti sweet compounds) the mixture of triterpene saponins, anthraquinone derivatives, alkaloids, betaine choline and trimethylamine [8]. Antisweet constituent of the leaves has been found to be a mixture of triterpene saponins. The sugar residues are glucuronic acid and galacturonic acid while ferulic and angelic acids have been attached as the carboxylic acid. Chewing of leaves reduces sensitivity to sweet substances [9].

The active principles which have been identified as glycosides (7 gymnemic acids) suggest that the topical and selective anaesthetic effect of the plant might result from the competition of the receptor sites between glycosides and the sweet substances [10]. In the present investigation an attempt has been made to evaluate *in vitro* antioxidant activity and preliminary phytochemical analysis of *Gymnema sylvestre* R.Br.

Correspondence:

Kalyani Singh

Regional Plant Resource Centre
(RPRC), Nayapalli, Bhubaneswar-
15, Odisha, India

2. Materials and Methods

2.1 Plant materials: Fresh leaves of *Gymnema sylvestre* R.Br. (with Field No - 4789/24.4.94) were collected from the botanical garden of Regional Plant Resource Centre (RPRC), Bhubaneswar, Odisha, India (Figure-1).



Fig 1: *Gymnema sylvestre* Plant

2.2 Preparation of Plant extracts: The leaves were dried under shade condition and powdered mechanically. 100 g of dry powdered leaf samples of *G. sylvestre* were extracted for 8 hours with three different solvents like ethanol, methanol and aqueous through soxhlet apparatus. The collected solutions were filtered through Whatman No-1 filter paper. The extract were evaporated to dryness under reduced pressure at 90 °C by Rotary evaporator and stored at -18 °C in a freeze until used for further analysis.

2.3 Preparation of Gymnema Stock Solution. Ethanolic, Methanolic and aqueous leaf extracts of *Gymnema sylvestre* were prepared at the concentration of 1,000 µg/ml. From the stock solution different concentration viz. 20, 40, 60, 80, 100 and 120 µg/ml were prepared for antioxidant studies and Ascorbic acid was used as standard for this study.

2.4 Qualitative Phytochemical analysis: Freshly prepared extracts were subjected to preliminary phytochemical analysis to find the presence of the followings phytoconstituents; alkaloids, cardiac glycosides, Anthraquinones, Tannins, Phenols, Terpenoids, Steroids, Saponins and Flavonoids. Qualitative tests were carried out using standard procedures to identify the constituents [11, 12, 13, 14].

2.4.1 Test for Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added [15]. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent [13]. The alcoholic extract was evaporated to dryness and the residue 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 samples were then observed for the presence of turbidity or yellow precipitation.

2.4.2 Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of concentrated sulphuric acid. A brown ring

of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.4.3 Test for Anthraquinones: Bomtregers test was used for the detection of anthraquinones. 5 gm of plant extract was shaken with 10 ml of benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet color in the lower phase indicated the presence of free hydroxyl anthraquinones.

2.4.4 Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

2.4.5 Test for Phenols: The solvent plant extract was treated with few drops of neutral ferric chloride solution 5% intense color developed which indicated the presence of phenols.

2.4.6 Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

2.4.7 Test for steroids: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H₂SO₄. The color changed from violet to blue or green in some samples indicating the presence of steroids.

2.4.8 Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.4.9 Test for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample [11, 14]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

2.4.10 DPPH Radical Scavenging Activity

Plant extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 20, 40, 60, 80, 100 and 120 µg/ml was added to 3 ml of a 0.004% ethanol solution of DPPH. An equal amount of ethanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance of the samples was measured at 517 nm. Radical scavenging activity was

calculated using the following formula:

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

The antioxidant activity of the extract was expressed as IC_{50} . The IC_{50} value was defined as the concentration (in $\mu\text{g/ml}$) 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^[16].

3. Results and Discussion

3.1 Qualitative Estimation of Phytochemicals:

Phytochemical analysis is very useful in the evaluation of

some active biological components of medicinal plants. The phytochemical screening carried on the leaves extract of *Gymnema sylvestre* revealed the presence of some active ingredients such as alkaloids, cardiac glycosides, tannins, saponins, anthraquinones, phenols and flavonoids (Table-1). This analysis determines the biologically active compounds that contribute to the flavour, colour and other characteristics of leaves. The phytochemical screening on qualitative level showed that the leaves of the plant *Gymnema sylvestre* were rich in alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity^[11].

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

Phytochemicals	<i>Gymnema sylvestre</i> leaf extract
Alkaloids	+
Cardiac glycoside	+
Anthraquinone	+
Tannins	+
Phenols	+
Terpenoids	--
Steroids	--
Saponins	+
Flavonoids	+
(+) denotes present and (-) denotes absent	

For the qualitative analysis results, below is the discussion. The research work that was carried out on the medicinal plant *Gymnema sylvestre* showed the presence of various types of phytochemicals constituents which were shown in Table-1. It shows that tannins, saponins, phenols, flavonoids, alkaloid, anthraquinone and Cardiac glycoside were present whereas steroid and terpenoids were found to be absent in *Gymnema sylvestre*. The presence of various active ingredients (secondary plant metabolites)^[17] as revealed by the phytochemical screening (Table-1) supports the resourcefulness of the plant extracts^[11, 18].

3.2 DPPH scavenging activity

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxy radicals, hydroxyl radicals and peroxy nitrile. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage^[19]. Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neuro degenerative diseases^[20].

Table-2 shows the results of the free radical (DPPH) scavenging activity in (%) inhibition. The result revealed that the ethanol fraction of *Gymnema sylvestre* exhibited the

highest DPPH radical scavenging activity with $83.41 \pm 0.13\%$ at $120 \mu\text{g/ml}$ concentration (which is nearly close to the value of Ascorbic acid i.e. $89.65 \pm 0.26\%$) followed by $66.28 \pm 0.11\%$, $52.22 \pm 0.17\%$, $40.33 \pm 0.16\%$, $31.17 \pm 0.18\%$ and $19.10 \pm 0.13\%$ at the concentrations of $100 \mu\text{g/ml}$, $80 \mu\text{g/ml}$, $60 \mu\text{g/ml}$, $40 \mu\text{g/ml}$ and $20 \mu\text{g/ml}$ respectively. Similarly in case of methanolic extract the highest inhibition activity i.e. $80.17 \pm 0.26\%$ was found at $120 \mu\text{g/ml}$ followed by $64.15 \pm 0.14\%$, $49.35 \pm 0.11\%$, $38.07 \pm 0.12\%$, $27.26 \pm 0.17\%$ and $15.02 \pm 0.23\%$ at different range of concentration ($100 \mu\text{g/ml}$, $80 \mu\text{g/ml}$, $60 \mu\text{g/ml}$, $40 \mu\text{g/ml}$ and $20 \mu\text{g/ml}$) respectively. The order of percentage of scavenging activity in case of aqueous leaf extract of *Gymnema sylvestre* were as follows: $72.11 \pm 0.18\%$, $60.31 \pm 0.19\%$, $45.21 \pm 0.36\%$, $33.37 \pm 0.31\%$, $24.23 \pm 0.11\%$ and $13.10 \pm 0.14\%$ at different concentration levels ($120 \mu\text{g/ml}$ - $40 \mu\text{g/ml}$) respectively (Table-2 and Figure-2).

The antioxidant capacity is also expressed as 50% inhibitory concentration (IC_{50}). A lower IC_{50} value means a higher antioxidant capacity of the sample. Significantly lowest IC_{50} value $76.59 \mu\text{g/ml}$ was observed in ethanolic extracts of *Gymnema sylvestre* which is close to $76.49 \mu\text{g/ml}$ obtained in the standard ascorbic acid.

Table 2: DPPH scavenging activity of *Gymnema sylvestre* R.Br. leaf extracted in different solvents

Concentration of extracts ($\mu\text{g/ml}$)	Antioxidant activity (%)			
	Ascorbic Acid	Ethanol	Methanol	Water
20	25.25 \pm 0.11	19.10 \pm 0.13	15.02 \pm 0.23	13.10 \pm 0.14
40	35.19 \pm 0.13	31.17 \pm 0.18	27.26 \pm 0.17	24.23 \pm 0.11
60	43.37 \pm 0.15	40.33 \pm 0.16	38.07 \pm 0.12	33.37 \pm 0.31
80	52.29 \pm 0.35	52.22 \pm 0.17	49.35 \pm 0.11	45.21 \pm 0.36
100	69.11 \pm 0.17	66.28 \pm 0.11	64.15 \pm 0.14	60.31 \pm 0.19
120	89.65 \pm 0.26	83.41 \pm 0.13	80.17 \pm 0.26	72.11 \pm 0.18

values represent means \pm SD, n=3

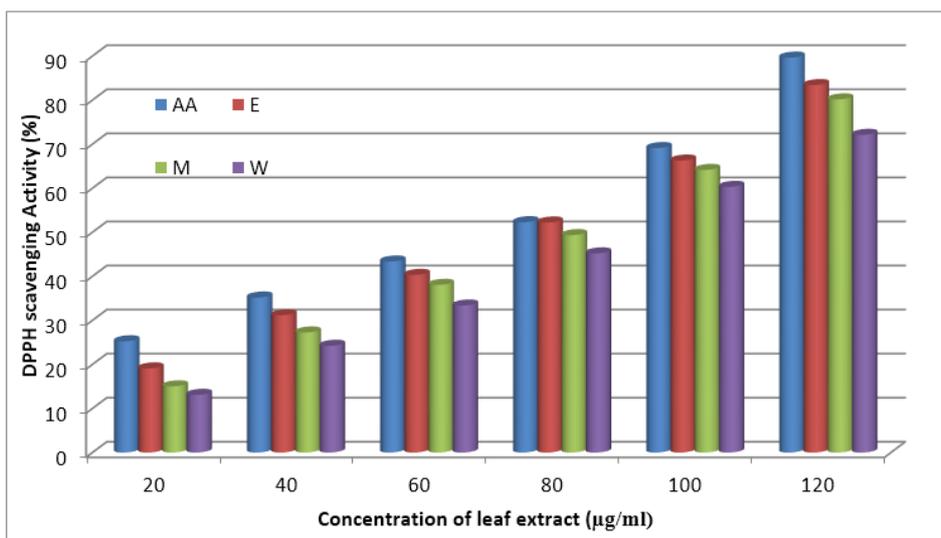


Fig 2: DPPH Radical Scavenging activity of *Gymnema sylvestre* leaf extract through different solvents
AA – Ascorbic acid, E – Ethanol, M – Methanol and W - Water

In overall comparison the ethanolic leaf extract of *Gymnema sylvestre* shows the highest scavenging activity followed by the aqueous and then methanol. Methanol and ethanol has been proven as effective solvent to extract phenolic compounds. Among solvents used in this study ethanol has showed the best effectiveness extracting phenolic components. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity [21]. It was observed that the antioxidant values were increased with increase in concentration of crude extracts which may be indicated that antioxidant values may be dependent on the presence of different phytochemicals such as alkaloids, flavonoids, saponins, tannins etc. It is reported that phenols are responsible for the variation in the antioxidant activity of the plant [22]. It has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as alkaloids, flavonoids, phenols and tannins [18, 23].

4. Conclusion

The present results revealed that the ethanolic leaf extract of *Gymnema. Sylvestre* R.Br. exhibited potent antioxidant activity by inhibiting DPPH free radicals which indicates the leaves of *Gymnema sylvestre* is very much rich in different types of phytochemical constituents especially alkaloids, tannins, saponins, phenols, glycosides, flavonoids etc. So it can be concluded that ethanolic leaf extract of *Gymnema Sylvestre* R.Br. can be used as an accessible source of natural

antioxidant agent.

5. Acknowledgement

The authors are thankful to the Director Regional Plant Resource centre (RPRC) for providing necessary facilities for this research work.

6. References

1. Bone K. Official home page of *Gymnema*: A key herb in the management of diabetes. <http://www.townsendletter.com>, 22 July, 2014.
2. Bouayed J, Piri K, Rammal H, Dicko A, Desor F, Younos C. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. *Food Chem* 2007; 104:364-368.
3. Burlon GW, Ingold KU. B-Carotene, an unusual type of lipid antioxidant. *J Sci* 1984; 224-573.
4. 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; 74:2157-2184.
5. Evans WC. Trease and Evans pharmacognosy, W.B. Saunders, Edinburgh, 2002, 540-541.
6. Harborne JB. *Phytochemical Methods*. Chapman & Hall, London 1973; 1, 49-188.
7. Hill AF. *Economic Botany. A textbook of useful plants and plant products*. Edn 2, McGraw-Hill Book Company Inc, New York, 1952.
8. Karadeniz F, Burdurulu HS, Koca N, Soyer Y. Antioxidant activity of selected fruits and vegetables grown in Turkey.

- Journal of Agriculture and Food Chemistry 2005; (29):297-303.
9. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Nirali Prakashan, Pune, Edn 36, 2006, 252.
 10. Kumaran A, Karunakaran JR. *In-vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India, LWT-Food Science and Technology, 2007; 40(2):344- 352.
 11. Lachman L, Lieberman HA, Kanig J. The Theory and Practice of Industrial pharmacy, Edn 3, Varghese publishing house, New York, 1989; 293-373.
 12. Mojab F, Kamalinejad M, Naysanch G, Hamid RV. Phytochemical screening of some species of Iranian plants. Iranian Journal of Pharmaceutical Research 2003; 2(2):77-82.
 13. Mondal SK, Chakraborty G, Gupata M, Mazumder UK. *In-vitro* antioxidant activity of *Diospyros malabarica* Kostel bark. Indian Journal of Experimental Biology 2006; 44:39-44.
 14. Nahak G, Sahu RK. Antioxidant activity of *Plumbago zeylanica* and *Plumbago rosea* belonging to Family Plumbaginaceae. Natural Products: An Indian Journal 2011; 7(2):51-56.
 15. Nahak G, Sahu RK. Phytochemical Evaluation and Antioxidant activity of *Piper nigrum* and *Piper cubeba*. Journal of Applied Pharmaceutical Science 2011; 01(08):153-157.
 16. Palozza P. Pro-oxidant actions of Carotenoids in Biologic Systems. Nutr Revolution 1998; 56(9):257-265.
 17. Rahman MAA, Moon SS. Antioxidant polyphenol glycosides from the Plant *Drabanemorosa*. Bull Korean Chem Soc 2007; 28(5):827-831.
 18. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trend Plant Sci 1997; 2:152-159.
 19. Siddique AA, Alim: Practical Pharmaceutical Chemistry. Edn 1. CSB Publishers & distributors, New Delhi, 1997; 126-131.
 20. Sofowora A. Medicinal Plants & Traditional medicine in Africa. New York: John Wiley & Sons, 1993, 191-289.
 21. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H, Phytochemical Screening and Extraction: A review: International Pharmaceutica Scientia 2011; 1(1):98-106.
 22. Trease GE & Evans WC: Pharmacognosy. Edn 11, Brailliar Tiridel Can. Macmillian Publishers, 1989.
 23. Trease GE & Evans WC: Pharmacognosy, Edn 15, Saunders, 214-393.
 24. Warren RP, Warren RM, Weninger MG. Inhibition of sweet taste of *Gymnema sylvestre*. Nature 1969; 223:94-95.
 25. Warriar PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants. Vol. 1-5. Orient Longman Ltd., Madras, 1993-1995.