IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF *GYMNEMA SYLVESTRE* R. BR. LEAF EXTRACT

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Preliminary phytochemical screening and *in-vitro* antioxidant activity of *Gymnema sylvestre* R. Br. leaf extract were investigated. The antioxidant activity was studied in some *in-vitro* antioxidant models like DPPH radical scavenging activity, superoxide radical scavenging activity, ferric reducing power and hydrogen peroxide scavenging activity. Total antioxidant capacity was also determined. The *Gymnema sylvestre* R. Br. alcoholic leaf extract showed antioxidant activity by inhibiting DPPH, scavenging superoxide and hydrogen peroxide. It also showed reducing power ability in ferric reducing model. Total antioxidant capacity was found to be 17.54 mg/g expressed as ascorbic acid. Significant antioxidant activity of alcoholic extract of *Gymnema sylvestre* R. Br. was found which might be due to the presence of Acidic compounds, Flavonoids, Phenols, Saponins, Tannis (Phenolic compounds) and Triterpenoids found in the preliminary phytochemical screening.

Key words: Antioxidant, gymnema, DPPH, Asclepiadaceae.

INTRODUCTION

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 (Mondal, 2006).

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 inducted 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 (Lachman, 1986). It has been suggested that fruits, vegetables, natural plants contain a large variety of substance called phytochemicals which are

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present in plants and are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexers of prooxidant metals, quenches of singlet oxygen etc (Ebadi, 2002). The antioxidants can interfere with the oxidation process by reacting with free radicals (Gupta, 2004). Recently interest has increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity (Kumaran, 2007). Antioxidants principles from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance (Shriwaikar, 2006). Food industry uses natural antioxidants as a replacement of conventional synthetic antioxidants (Govindarajan, 2003).

Gymnema sylvestre R.Br. (Asclepiadaceae) a large woody, much branded, climber with pubescent young parts, found throughout India in a dry forest up to 600m, Deccan peninsula leaves opposite, usually elliptic or ovate (The Wealth of India, 2005). Leaves contain lupeol, β-amyrin, stigmasterol, pentriacontane, hentricontane, α and β chlorophyll, resin, tartaric acid, gymnemic acid (anti sweet compounds) the mixture of triterpene saponins, anthraquinone derivatives, alkaloids, betain, choline and trimethylamine (Kokate, 2006).

In the Ayurvedic system of medicine, *Gymnema sylvestre* R.Br. is referred to as "mesasrngi," and both the dried leaf (mesasrngi leaf) and dried root (mesasrngi root) are used therapeutically. The leaves of the plant in particular are used as antiviral, diuretic, antiallergic, hypoglycemic, hypolipidemic, for the treatment of obesity and dental caries (The Ayurvedic Pharmacopoeia of India, 2006). It is also used as Antibiotic, in stomach pains, as a blood purifier and in rheumatism (Evans, 2002). As far as our literature survey could ascertain, there is no *in vitro* study reported for antioxidative activity of leaf extract *Gymnema sylvestre* R.Br. Therefore, the aim of the study was *in vitro* evaluation of antioxidant activity of *Gymnema sylvestre* R. Br. leaf extract.

MATERIAL AND METHODS

Chemicals. Ascorbic acid, rutin, gallic acid, hydrogen peroxide, potassium ferricyanide, trichloroacetic acid, ferric chloride, folin-ciocalteu reagent, indigosulphonic acid, α-α diphenyl β picryl hydrazyl (DPPH), Riboflavin**,** Nitro Blue Tetrazolium (NBT) and Dimethyl Sulphoxide (DMSO) were all purchased from SD-fine chemicals, India, all other reagents used were of analytical grade.

Instrument. UV spectrophotometer (Shimadzu-UV-1601), Centrifuge Machine (Eltek-research centrifuge-TC-4100D).

Plant Material. The leaves of *Gymnema sylvestre* R.Br. were collected locally from the college campus of Modasa-Gujarat (India) and were authetified by Dr. M.S.Jangid, Botany Department from Sir P.T Science College-Modasa, India. Authentication specimen number is PRR/01/01012008.

Extraction of Plant Material. The Gymnema (Gurmar) leaves are graded, cleaned and disintegrated to required mesh (20-60). It is then extracted with 55% v/v alcohol using soxhlet apparatus for 6 hours. The micella is concentrated under reduced pressure to 30% solids. It is filtered, weighed and used for the study (Rajpal, 2002).

Preliminary Phytochemical Screening. Preliminary phytochemical screening of the Gymnema leaf extract was carried out for the detection of the various plant constituents (Khandelwal, 2004).

Preparation of Gymnema Stock Solution. Alcoholic extracts of gymnema was prepared at the concentration of 1,000 µg/ml in methanol. From the stock solution different concentration viz. 10, 20, 40, 60, 80 and 100 µg/ml were prepared in methanol and used for antioxidant studies.

Preparation of Standard Stock Solution of Ascorbic Acid. Ascorbic acid used as standard for the study and its stock solution was prepared in the concentration of 1,000 µg/ml in methanol. It was prepared freshly and used immediately for the study. From the stock solution different concentration viz. 10, 20, 40, 60, 80 and 100 µg/ml were prepared in methanol and used for antioxidant studies.

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 (Kumaran, 2007).

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 (Kumaran, 2007).

Superoxide Radical Scavenging Activity. Each 3ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, and 12 mM EDTA and 0.1 mg NBT and 1ml of sample solution. Reaction was started by illuminating the reaction mixture with different concentrations of plant extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml for 5min. Immediately after illumination, the absorbance was measured at 590 nm. Identical tubes with reaction mixture and 1ml of methanol were kept in the dark along and served as control. The percentage inhibition of superoxide anion generation 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}. All the tests were performed in triplicate and the graph was plotted with the average of three observations (Kumaran, 2007).

Scavenging Of Hydrogen Peroxide. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of plant extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml in methanol (1 ml) where added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. 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 extract/standard. The antioxidant activity of the extract was expressed as IC_{50}. All the tests were performed in triplicate and the graph was plotted with the average of three observations (Kumaran, 2007 and Srinivasan, 2007).

Ferric Reducing Power Determination. Different concentrations of plant extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50° C for 20 min. A portion (2.5 ml) of tricholoroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 g (rpm) for 10 min at room temperature. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride ($FeCl_3$) (0.5 ml, 0.1%) and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700 nm. All the tests were performed in triplicate and the graph was plotted with the average of three observations (Kumaran, 2007 and Kumar, 2005).

Statistical Evaluation. Experimental results were mean±SEM of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value. Student's t-test was used for the comparison between two means for the possible significant interrelation. Data were considered statistically significant only when p value < 0.05.

RESULTS

Total Antioxidant Capacity. The total antioxidant capacity in the gymnema leaf extract measured spectrophotometrically was 17.54 mg/gm expressed as ascorbic acid.

DPPH Radical Scavenging Activity. Fig. 1 illustrates a significant ($p < 0.05$) decrease in the concentration of DPPH radicals due to the scavenging ability of gymnema extract. This activity was dose dependent. Maximum scavenging activity (57.10%) was observed at 100 µg/ml concentration and the IC_{50} value of gymnema extract and Ascorbic acid were found to be 85.28 µg/ml and 41.41 µg/ml respectively.

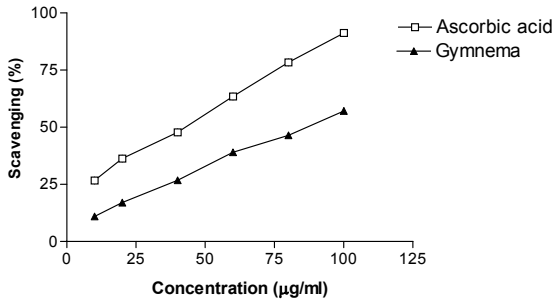

Fig. 1. DPPH radical scavenging activity of gymnema extract.

Super Oxide Radical Scavenging Activity. Fig. 2, reveals that a significant ($p < 0.05$) dose response relationship is found in the superoxide free radical scavenging activity in gymnema extract. Maximum scavenging activity (65.29%) was observed at 100 µg/ml concentration of and the IC_{50} value of gymnema extract and ascorbic acid were found to be 71.82 µg/ml and 35.99 µg/ml respectively.

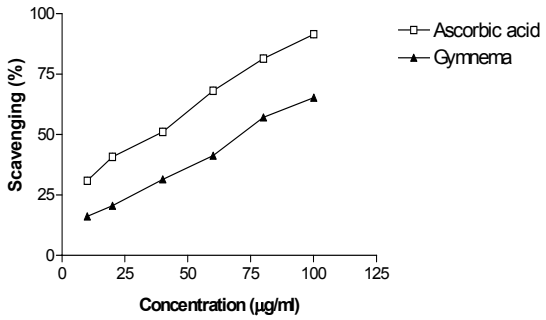

Fig. 2. Superoxide radical scavenging activity of gymnema extract.

Scavenging of Hydrogen Peroxide. Fig. 3 reveals that a significant ($p < 0.05$) dose dependent response was found in the hydrogen peroxide scavenging activity in gymnema extract. Maximum scavenging activity (65.26%) was observed at 100 µg/ml concentration and the IC_{50} value of gymnema extract and ascorbic acid were found to be 72.55 µg/ml and 39.84 µg/ml respectively.

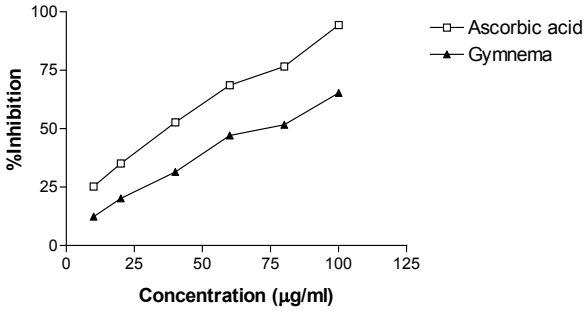

Fig. 3. Hydrogen peroxide radical scavenging activity of gymnema extract.

Ferric Reducing Power Determination. Fig. 4 reveals that reducing power of gymnema extract was statistically significant ($p < 0.05$). The result clearly indicates that the reducing power of the gymnema extract increased with increasing the concentration and is comparable with the standard ascorbic acid, hence it is having the antioxidant activity.

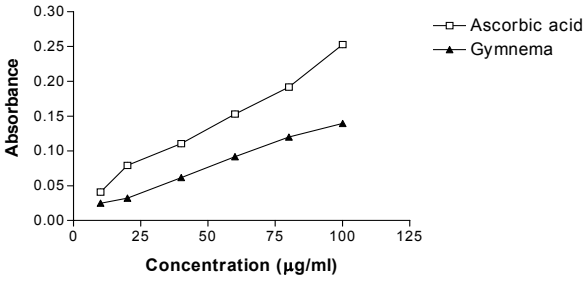

Fig. 4. Reducing power of gymnema extract.

DISCUSSION

The total antioxidant capacity in the gymnema extract was determined by the the formation of the phosphomolybdenum complex. The gymnema extract was able to reduce the stable radical DPPH to the yellow coloured

diphenylpicrylhydrazine, it was also found to be an efficient scavenger of superoxide radical generated in riboflavin-NBT-light system *in-vitro* and their activity are in comparable to that of ascorbic acid. Photochemical reduction of flavins generates oxygen radicals which reduces NBT, resulting in the formation of blue formazan. Gymnema extract inhibits the formation of blue formazan and it also scavenges the toxic hydroxyl radicals generated by hydrogen peroxide. The reductive ability was found from the Fe^{3+}–Fe^{2+} transformation in the presence of the gymnema extract.

CONCLUSION

The gymnema extract showed antioxidant activity by inhibiting DPPH, scavenging super oxide as well as hydrogen peroxide and reducing power ability which may be due to presence of Flavonoids, Phenols, Tannis (Phenolic compounds) and Triterpenoids found in the preliminary phytochemical screening.

Thus, the radical scavenging activity suggests that 55% v/v alcoholic extract of *Gymnema sylvestre* R. Br. *In-vitro* antioxidant activities. Further studies are needed to evaluate the *in-vivo* antioxidant potential of gymnema extract in various animal models.

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