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Evaluation of free radical scavenging capacity of gymnemic acid isolated from *Gymnema sylvestre* leaves.

Sankaradoss Nirmala[1] and Velayutham Ravichandiran[2]

[1]Department of Pharmacognosy, School of pharmaceutical sciences, Vels University India
[2]National institute of pharmaceutical education and Research, Kolkatta, India

Abstract : The gymnemic acid fraction has various triterpenoids, terpenoidal glycosides and saponins has been receiving much attention for its free radical scavenging and antioxidant capacity. Free radical scavenging capacity of enriched gymnemic acid fractions were investigated by DPPH, β-carotene linoleic acid, hydroxyl and nitrates radicals inhibition properties. The results from DPPH reveals that gymnemic acid fraction showed efficient quenching of DPPH* the fractions thus contain free radical quenching compounds, with act as primary radical scavenging that react with DPPH* by providing a hydrogen atom or electron donating ability). The gymnemic acid fraction have shown the inhibition of the coupled oxidation of the linoleic acid and β-carotene in emulsified aqueous system. This may be explained by the radical scavenging capacity of fraction and its inhibitory action on nitric oxide and hydroxyl radicals may contribute to some extent to the β-carotene assay. The present data explain the multiple free radical scavenging capacity and antioxidant capacity of gymnemic acid fraction of Gymnema sylvestre leaves as compared with standard antioxidant Ascorbic acid. A further gymnemic acid fraction has shown good inhibition in scavenging nitrates which is generated in *in-vitro.*
Key words : Antioxidant, Gymnemic acid, DPPH, β-carotene, Ascorbic acid.

1. Introduction:

Antioxidants or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc.[1,2] Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders .[3]

Antioxidant acts as a major defence against radical-mediated toxicity by protecting the damages caused by free radicals. The defence system to combat the potentially damaging effects of free radical species includes enzymes such as catalase, glutathione peroxidase, superoxide dismutase and non enzymes such as ascorbic acid, α-tocopherol and uric acid [4].Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be involved in a high number of diseases [5]. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. Ayurveda, the Indian traditional health care system (ayus_ life, veda_knowledge, meaning science of life), is the oldest medical system in the world and is being revived in its complete form under the name of Maharishi Ayurved . Several medicinal plants have been used as dietary adjunct and in the

treatment of numerous diseases without proper knowledge of their function. The essential value of some plants has long been published and the large numbers of them remain unexplored as yet. One such plant is *Gymnema Sylvestre* is a woody, climbing plant of tropical forests of central and southern India and in parts of Africa. Gymnema has been referred to in some texts as *Asclepias geminata, Gymnema melicida,* and *Periploca sylvestris*. Gymnema has played an important role in Ayurvedic medicine for centuries. The major class of phytochemical belongs to *G. sylvestre* leaves contains triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are gymnemasides. The other chemical constituents are flavones, anthraquinones, hentri-acontane, pentatriacontane, á and â-chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, â-amyrin related glycosides and stigmasterol, some alkaloids and anthroquinones. The folklore claim of gymnema sylvestre leves are Antiflu, Antihistaminic, Antiinflammatory, Antiobesity, Antidiabetic Antipyretic, Antiseptic, Antiviral, Cyclooxygenase-Inhibitor, Fungicide, Gastrostimulant, Hypotensive, Hypothermic, Immunostimulant, Molluscicide, Mutagenic, Nematicide, Progesteronigenic, Sedative, Serotoninergic, Thyrotropic[6]. Hence, the objective of the present study was designed to investigate the free radical scavenging capacity of gymnema sylvestre leaves in various in-vitro models.

2. Materials and Methods

2.1 Collection and authentication

Gymnema sylvestre leaves were collected from Anna Herbal Garden, Chennai, Tamil Nadu. It was identified by Botanist, Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu and specimen voucher (**PARC/2012/1279**) are kept at Department of Pharmacognoscy, Vels College of Pharmacy, Chennai. The leaves of *Gymnema sylvestre* were shade dried grounded and stored dry until extraction.

2.2 Extraction of Gymnemic acid by Hoopers method[7]

Step 1: extraction with petroleum ether (Defatting process)

I kg of dried *gymnema sylvestre* dry leaf powder was packed into a clean soxhlet extraction unit. Seven litres of petroleum ether (60-80°C) was added and extracted for 24-36 hrs till all components are soluble in petroleum. petroleum ether extract is collected and distilled . Then a net of 240gm of petroleum extracts was obtained. petroleum ether extracts was obtained.

Step 2: Extraction with 90% methanol

The plant material is then extracted with 90% methanol. 90% methanol is added and the extraction was carried out for 24-36hrs till total methanol soluble extracts was obtained. Then methanol soluble extract was distilled and finally 185gm of thick paste were obtained.

Step 3: Isolation of pure gymnemic acid from methanol extract

175gm thick paste of methanol soluble extract was dissolved in 1% aq.KOH solution on continuously stirring for 45 min to 1hr.The solution is then filtered through filter paper to separate the undissolved particles. Diluted HCL was added slowly under constant stirring, during which the gymnemic acids were precipitated. Precipitated solution was filtered under suction and precipitate was dried. The pure gymnemic acid was obtained. The yield of crude gymnemic acid fraction was found to be 29.6%. The isolated gymnemic acid fraction was subjected to qualitative chemical test and thin layer studies and positive tests for steroids, terpenoids and glycosides. The gymnemic acid fraction was dissolved in ethanol used for further studies .

2.3 Thin layer chromatography(TLC):

The identification and seperation of components present in crude gymnemic acid fraction was carried out by thin layer chromatography. The TLC of gymnemic acid was performed using different solvent systems i.e chloroform; acetone, chloroform;methanol, toluene;ethyl acetate, ethyl acetate;diethylamine, ethyl acetate;petroleum ether. The chromatograms were dried to remove the solvent, cooled and sprayed with detecting reagents. The plates were dried at 105°c for 5min to enable the full colour of spots to develop. From the thin layer chromatographic studies, the presence of various compounds was observed with Rf values

between 0.66 and 0.80. The Rf value of gymnemic acid present in leaves of gymnema sylveestre was compared with standard gymnemic acid Rf value 0.71 and gave a good agreement . Further the gymnamic acid fraction was labeled as GAF.

2.4 Chemicals and drugs

Ferrous sulphate, Ascorbic acid, Deoxyribose, Trichloro acetic acid (TCA) and Thio barbituric acid (TBA) linoleic acid,β-carotene were obtained from Sisco Research Laboratory (SRL), Mumbai, India 1, 1-dipheny1-2-picry1-hydrazy1 (DPPH) was procured from Sigma-Aldrich Co MO, USA. Other chemicals used were of analytical grade. The gymnemic acid fraction isolated from gymnema sylvestre leaves.

2.5 DPPH radical scavenging capacity

The effect of gymnemic acid fraction on DPPH radical was estimated by [8] with minor modification

n. In brief, 2mL of DPPH in methanol (3.6 x 10^{-5} M) were added to 50 µL of various concentrations of test substance (25µl -1ml). The mixture was vortexed for 15 sec and left to stand at 37^{0}C for 30 min. The decrease in the absorbance at 515 nm was continuously recorded in a spectrophotometer for 15 min at room temperature. All determination was performed in triplicate. The DPPH scavenging activity (decrease of absorbance at 515 nm) of extract was plotted against time and the (%) percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 15 min duration as follows,

% Inhibition = [$Abs_{Control}$ – Abs_{Sample}]/ $Abs_{Control}$ x 100

Where $Abs_{Control}$ is absorbance of control at time = 0 and Abs_{Sample} is absorbance of test sample at time = 15 min.

2.6 β- Carotene-Linoleic acid (Linoleate) Assay

The antioxidant capacity is measured by the ability of a compound to minimize the coupled oxidation of linoleic acid and β-carotene in an emulsified aqueous system, which loses its orange color when reacting with the radicals [9]. In this method 2ml of βcarotene-linoleate emulsion(20mg/ml βcarotene/0.2ml of linoleic acid,5ml of tween40 and 50ml of distilled water are added with vigorous stirring and saturated with oxygen)is equilibrated at 32˚c for 5min.the oxidation reaction is initiated by adding 10µl APPH (0.9M) (2,2′-azobis(2-methyl propinomide)dihydrochloride) with 10 min vortexing the mixture. To this mixture various concentration (0.1Mm to 1Mm) of test gymnemic acid fraction samples are added and the mixture is vortexed again for few min. the absorbance of sample is measured at 470nm, immediately after sample preparation (t=0min) and at 15min interval until the end (t=120min) of the experiment. The percent inhibition rate of β-carotene bleaching relative to the control was calculated by the formula

% Antioxidant capacity (AOA) =1-[Abs_{sample}t = 0min-Abs_{sample}t = 60or 120min] x 100

[$Abs_{control}$t = 0min-$Abs_{control}$t =60or120min]

2.7 TBARS Assay

Oxidation reaction was performed with 1ml volume of PBS, 0.5 mM $Cacl_2$, PH 7.4 and with purified LDL at a concentration of 50 µg/ml and oxidation of LDL was catalyzed 2 µM $Cucl_2$, either before or oxidation in the presence or absence of bovine serum albumin (BSA) and 500 µl of 20% Tri chloroacetic acid (TCA) were added and the reaction mixture was placed on the ice for 5 min after precipitation, 500 µl of a 1% thiobarbituric acid (TBA) solution were added, and the samples were heated at 95° C for 45min, and cool down on ice for another 5 min and centrifuged at 1000 X g for 20min, subsequently the absorbance of the supernatant was measured at 532 nm [10].

2.8 Nitric oxide scavenging assay

Nitric oxide scavenging assay was measured by spectrophotometric ,method described was mixed with different concentration of extract and fraction dissolved in methanol and incubated at 250c for 30 min. After 30 min 1.5 ml of the incubation solution were removed and diluted with 1.5 ml of modified Griess reagent

(sulphanilic acid with naphylethylene diamine dichloride in acetic acid). The absorbance was measured at 546 nm. [11]

% Inhibition = [Abs $_{Control}$ – Abs $_{Sample}$] /Abs control x100

2.9 Deoxyribose protection against hydroxyl radicals

Deoxyribose protection against hydroxyl radical (OH*) generated by reacting Fe^{3+} , EDTA, ascorbic acid and H_2O_2 [12]. In brief, 1 ml of final reaction solution contained 500 µ1 of various concentration of test materials, $FeC1_3$ (100 µM), EDTA (100 µM), H_2O_2 (1 mM), deoxyibose (3.6 mM) abd ascorbic acid (100 mM) in potassium phosphate buffer (KH_2PO_4-KOH, pH 7.4). Deoxyirbose degradation by hydroxyl radical was measured by using the thiobarbituric acid method. The reaction mixture was incubated for 1 h at 37^0C and further heated in a boiling water bath for 15 min after addition of 1 ml TCA (10% w/v) and 1 ml of TBA (0.5% w/v) and color produced was measured at 532 nm against a blank containing phosphate buffer. Ascorbic acid was used as positive control. The % of OH* radical inhibition was calculated by following formula.

% Inhibition = [Abs $_{Contro}$l – Abs $_{Sample}$] / Abs $_{Control}$ x 100

3. Results

3.1 Effect of gymnemic acid fraction from gymnema sylvestre in DPPH radical scavenging assay

Figure 6.1 depicts the free radical scavenging capacity of gymnemic acid fraction and gymnemic acid gold nanoparticle using DPPH generated radical in *in-vitro*. It was observed that increase in % inhibition of free radicals has observed in increasing concentration of GAF . The IC_{50} value of GAF was found to be 39.23µg/ml the R^2- linear regression value was found to be 0.9968. The fractions was compared with the standard Ascorbic acid IC_{50}-59.52µg/ml;R^2-0.9987.

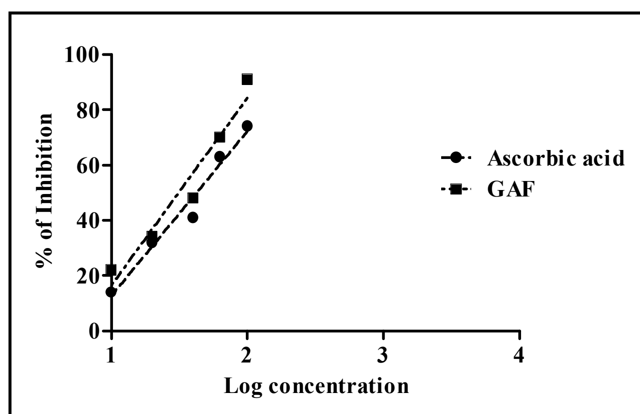

Fig 6.1 Effect of gymnemic acid fraction from gymnema sylvestre in DPPH radical scavenging assay

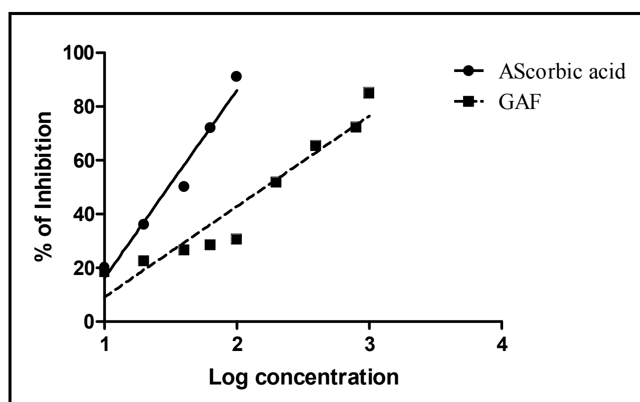

Fig 6.2 Effect of gymnemic acid fraction from gymnema sylvestre in β-carotene-Linoleic acid (Linoleate) assay

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Fig 6.2 Inhibition of β-carotene results are shown in figure 6.1. Dose dependent inhibition of β-carotene was noted in addition of various concentrations (10µl – 100µl) of gymnemic acid fraction and gymnemic acid gold nanoparticle. The IC_{50} value of gymnemic acid were 353.3µg/ml and the R^2 values were 0.9821 respectively which is comparable to that of IC_{50} and R^2 value of standard Ascorbic acid (126.1µg/ml and 0.9939).

3.3 Effect of gymnemic acid fraction from gymnema sylvestre measured by thiobarbituric acid in-vitro

Figure 6.3 depicts the %inhibition gymnemic acid fraction against LPO measured as a indicator of LDL oxidation. It is observed from the figure that addition of increasing concentration of gymnemic acid fraction increase the inhibit of LDL oxidation. In the inhibitory effect of gymnemic acid fraction % inhibition of LDL oxidation was found to be IC_{50} value was found to be 96.29 tested in linear regression analysis and corresponding R^2 was found to be 0.9950 respectively compared with standard Ascorbic acid IC_{50} value of 196.8µg/ml and R^2 value of 0.9610.

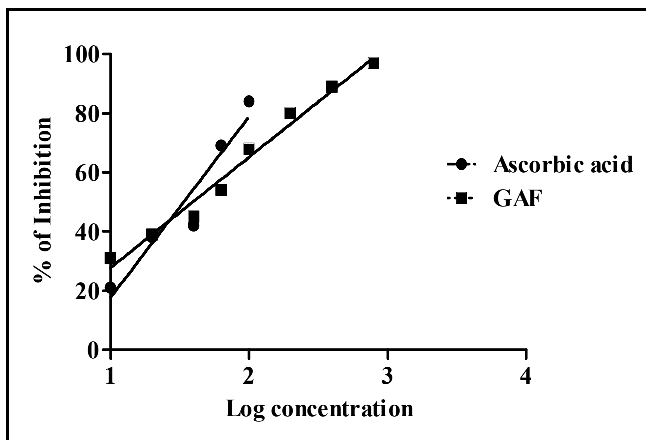

Fig 6.3 Effect of gymnemic acid fraction from gymnema sylvestre measured by thiobarbituric acid in-vitro

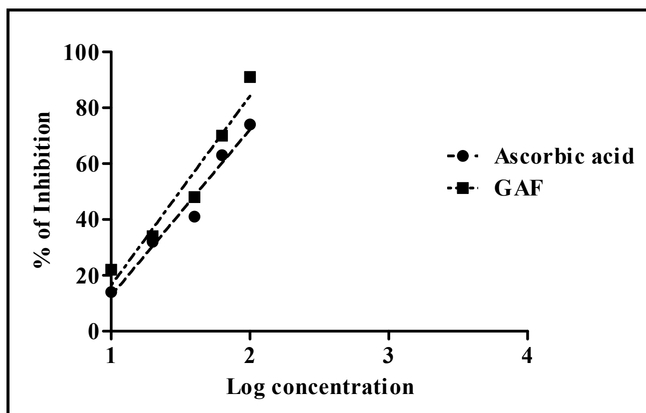

Fig 6.4 Effect of gymnemic acid fraction from gymnema sylvestre in nitric oxide scavenging:

3.4 Effect of gymnemic acid fraction from gymnema sylvestre in nitric oxide scavenging:

Figure 6.4 depicts the ability of gymnemic acid fraction to quench NO radicals was tested *in-vitro*. The results indicates that gymnemic acid fraction exhibited IC_{50} and R^2 values of 229.8µg/ml and 0.9963 respectively compared with standard Ascorbic acid IC_{50} value of 69.41µg/ml and R^2 value of 0.97ss76.

3.5 **Effect of gymnemic acid fraction from Gymnema sylvestre in deoxyribose protection against hydroxyl radicals.**

Figure 6.5 depicts the ability of of gymnemic acid fraction to quench hydroxyl radicals was tested *in-vitro*. The results indicates that of gymnemic acid fraction exhibited hydroxyl radical quenching with their IC_{50} and R^2 value was found to be 75.98µg/ml and 0.9727 respectively. The both fractions were compared with standard Ascorbic acid with IC_{50} value of 191.2 µg/ml and R_2 value is 0.9710.

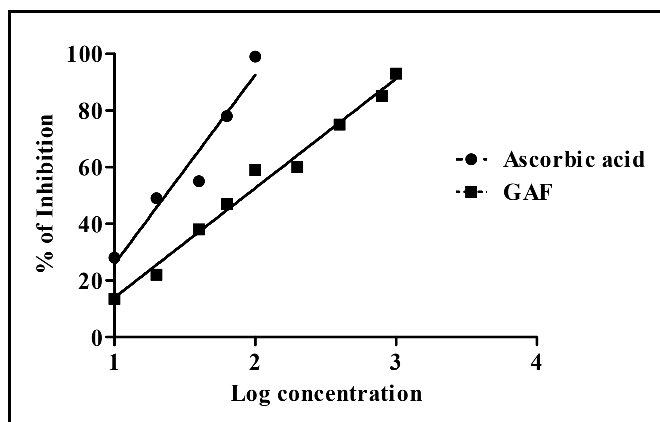

Fig 6.5 Effect of gymnemic acid fraction from Gymnema sylvestre in deoxyribose protection against hydroxyl radicals.

4. Discussion

There is an increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants[13,14]. Free radical scavenging capacity of gymnemic acid fraction is measured to inhibit various invitro methods. . The tested plant extracts showed strong antioxidant activity or differential capacity to inhibit DPPH, Nitric oxide, β-carotene method and TBARS .

Keeping in view its high antioxidant property, this plant can also be used alone or in combination in the form of different herbal formulations to protect the body from deleterious effects of free radicals. The results from DPPH reveals that gymnemic acid showed efficient quenching of DPPH* the fractions thus contain free radical quenching compounds, with act as primary radical scavenging that react with DPPH* by providing a hydrogen atom or electron donating ability. There are several other mechanisms by which antioxidants can act. One of them is by scavenging of reactive oxygen and nitrogen free radicals. There are many different experimental methods by which the free radical scavenging activity can be estimated. It has been reported that mitochondrial metabolism represents a major source of ROS such as nitric oxide and hydroxyl and hydrogen provides. The gymnemic acid exhibited inhibition of nitric oxide, hydroxyl radical using of deoxyribose as source, which explain the capacity of gymnemic acid fraction to react with one oxidant with either organic radical or redox active compounds. A further gymnemic acid fraction has shown good inhibition in scavenging nitrates which is generated in *in-vitro.*

References

1. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and diseases. Biochem J.1984;219:1–4. [PMC free article] [PubMed].
2. Maxwell SR. Prospect for the use of antioxidant therapies. Drugs. 1995;49:45–361.
3. Rice-Evans CA, Miller NJ, Paganga G. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20:933–56. [PubMed]
4. Sankaradoss Nirmala, KF Haja Nazeer ahamed, Velayudem Ravichandiran "Comparative in vitro study on the free radical scavenging capacity of tannin and biflavone fraction from Ficus racemosa Linn and Araucaria bidwilli Hook" International Journal of Chem Tech Research 2011. Vol. 3, No.3, pp 1440-1445.

5. Md. Sarfaraj Hussain , K. F. H Nazeer Ahamed, V. Ravichandiran, Md. Zaheen Hassan Ansari, Evaluation of in vitro free radical scavenging potential of different fractions of Hygrophila auriculata (K. Schum) Heine., Asian Journal of Traditional Medicines, 2009, 4 (5).
6. R. Balamurali krishna, Sujitha r. reddy, Harika javangula , D. Swapna, K. Jagadeeswara reddy An easy and simple method of isolation and purification of genomic DNA from the leaves of Gymnema sylvestre-an anti-diabetic plant., International journal of life sciences and pharma research,2012 ,2(1)Pp 15-20.
7. Hooper, D. Isolation and antiviral activity of gymnemic acid (1887). Pharm. J. Trans. 17:867-868.
8. Lim KT, Hu C, Kitts DD. Antioxidant activity of a *Rhus verniciflua* stokes ethanol extract. Food chemistry and Toxicology 2003; 39: 229-237
9. Unten, L., Koketsu, M., & Kim, M. (1997). Antidiscoloring activity of green tea polyphenols on b-carotene. Journal of Agricultural and Food Chemistry, 45, 2009–2019.
10. Miguel, G., Simo˜es, M., Figueiredo, A. C., Barroso, J. G., Pedro, L. G., & Carvalho, L. (2004). Composition and antioxidant activities of the essential oils of Thymus caespititius, Thymus camphoratus and Thymus mastichina. Food Chemistry, 86, 183–188.
11. Sreejayam N, Rao MNA. Nitric oxide scavenging activity of curcuminoids. Journal of Pharmacy and Pharmacology 1997; 49: 105
12. Halliwell B. Free radicals, antioxidant and human disease curiosity or consequence? The Lancet 1994;344: 721-724
13. Osawa T. Postharvest biochemistry. In: Uritani I, Garcia VV, Mendoza, EM, editors. Novel neutral antioxidant for utilization in food and biological systems. Japan: Japan Scientific Societies Press; 1994. p. 241-251.
14. Noda Y, Anzai-Kmori A, Kohono M, Shimnei M, Packer L. Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectromoter system. Biochem. Mol. Biol. Inter. 1997; 42 : 35-44.

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