PhytochemicalAnalysisandAntioxidant activity of Aqueous Fruit Extract of *Gymnema sylvestre*

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Abstract- *Gymnema sylvestre Wild R.Br belongs to family Apocynaceae (Subfamily: Asclepiadaceae), an herbal medicinal plant used in traditional medicine to treat diabetes. This plantis woody climber found in central and peninsular India. The plant is considered to be a good source of a large number of bioactive substances.The qualitative phytochemical analysis of fruit water extract shows presence of Steroids, tannins, saponins, flavanoids, carbohydrates, glycosides, terpenoids, phenolics, reducing sugars,fixed oils and fats etc.In-vitroantioxidant activity of the water extract of G. sylvestre fruit was studied using different antioxidant methods.Antioxidant activity of Fruit extracts which was comparable with that of BHT in DPPH, β-carotene bleaching and ABTS radical scavenging assays. The total phenolic content of Fruit extract is 80 mg GAE / g dry weight. The results of studies showed that the extract has considerable antioxidant potential and source of phytochemicals.*

Keywords- Gymnema sylvestre,Fruit,Phytochemical analysis,Antioxidant activity.

I. INTRODUCTION

G. sylvestre (Asclepiadaceace), a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India.The plant belongs to family Apocynaceae (Subfamily: Asclepiadaceae) is considered to be a good source of a large number of bioactive substances. *G. sylvestre* leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are Gymnemoside. Besides this, other plant constituents are flavones, anthraquinones, hentriacontane, pentatriacontane, α and β-chlorophylls, phytin, resins, dquercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigmasterol [1].The major constituents of leaves of *G. sylvestre* aregymnemic acids molecule and it was successfully isolated, purified and studied for antidiabetic array[2, 3]. Antimicrobial activity ofaqueous, methanolic and ethanolic extract of *G. sylvestre* leaves was reported earlier[4]. It is evident from the available literature that some of the antidiabetic plants possess antioxidant activity. Antioxidant activity of alcoholic leaf extract [5] and antibiotic activity of *G. sylvestre* extracts [6] was reported. Also, there are number of reports on antimicrobial activities of *G. sylvestre* [7, 8].*Gymnema sylvestre* leaf essential oil was also tested for antioxidant activity [9]. In previous studies antioxidant and antimicrobial activities of Leaf, stem and root of *G.Sylvestre* were reported[10,11]. Literature survey indicates that the antioxidant and antimicrobial properties of the *G. sylvestre* fruit is hitherto unknown. A study was therefore undertaken to evaluate antioxidant activity of fruit extract (FE) of *G.Sylvestre*.

II. MATERIALS AND METHODS

1. **Plant material:** The plants of *G. sylvestre* were collected from 'Pune'Maharashtra, India. The plant was authenticated by Botanical Survey of India, Pune (BSI). The material has been deposited at AHMA herbarium at BSI (Voucher No.SVS-1/783).

2. **Chemicals:** Butylated hydroxy toluene (BHT), tween-20 and Ammonium persulphate were purchased from Loba Chemicals, linoleic acid was purchased from SRL, β-carotene from HIMEDIA and Folin-Ciocalteu reagent was purchased from Qualigens. These are Indian companies. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azinobis-(-3-ethyl benzothiazoline-6-sulphonic acid) diamonium salt (ABTS), ammonium persulphate, were purchased from Fluka, USA. All the solvents used were of analytical grade.

3. **Preparation of extract:** Dried and Powdered Plant material (100g) was placed in 2 Lit. Conical flask and distilled n-hexane was poured into it. The mixture was kept overnight at room tempeature. Dark Greenish Coloured solution was filtered and fresh n-hexane was

again added to plant material. This process was continued till the n-hexane solution was colourless. The Powdered residue obtained is then subjectedfor further extraction by using Distilled water. The extraction with distilled water was carried out overnight at room tempeature. Dark brownish Coloured solution of distilled water was decanted. This process was continued till the n-hexane distilled water above the plant material becomes colourless. Finally powdered residue is then subjected to 1 lit.ofwater: ethanol (1:1) extraction. The material is extracted with three times by using water: ethanol (1:1) solvent system. Finally water extract and water ethanol extract were combined and concentrated under reduced pressure. The obtained water extract is subjected for phytochemical analysis and antioxidant activity evaluation. .

4. Qualitative Phytochemical Analysis of *G.Sylvestre*

The qualitative phytochemical analysis of the extracts was done using standard procedure as described in [12, 13, 14, 15]. The following qualitative tests were carried out as follows.

a. **Steroids:** 10 mg of the extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of conc. sulphuric acid. Blue colour in chloroform layer which changes to green shows the presence of steroids, whereas the appearance of pink colour in chloroform layer shows presence of terpenoids.
b. **Test for fixed oils and fats:** Small quantity of the each extract was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for1-2hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.
c. **Terpenoids:** To 0.5 gram of plant extract was added to 2 ml chloroform. Concentrated sulphuric acid (3 ml) was added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoids.
d. **Alkaloids:** The aqueous extract 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 sample was observed for turbidity or yellow precipitation.
e. **Flavonoids:** The 4 ml of extract was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloric acid was added and red colour was observed for flavonoids and orange color for Flavones.
f. **Coumarins:** 10 gram of the extract is dissolved in methanol and alcoholic KOH was added. The appearance of Yellow colour which decolorizes while adding Conc. HCl shows the presence of Coumarins.
g. **Saponins:** To 0.5 gram of extract was boiled in 10 ml water in test tube. The solution was shaken vigoursly and observed for persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, after which it was observed for the formation of emulsion
h. **Tannins**: About d and observed for brownish green or a blue black colouration.0.5 gram of the extract was boiled in 10 ml water in test tube and the filtered. A few drop of 0.1 % Ferric Chloride was added.
i. **Phenolic Compounds:** Extract was dissolved in alcohol and 1 drop of neutral ferric chloride was added to this. The intense colour indicated the presence of phenolic compounds.
j. **Anthraquinones**: To the extract Magnesium acetate solution was added the pink colour developed indicates the presence of Anthraquinones.
k. **Quinone:** Few mg of the Extract in alcohol is treated with sulphuric acid. The colour developed indicates the presence of quinones.
l. **Catechin:** Few drops of extract is treated with a few drops of Ehrlish reagent and few drops of Concentrated HCl. The pink colour developed indicates the presence of Catechin.
m. **Reducing Sugar:** Aqueous extract was added to boiling Fehling's solution (A and B). The solution was observed for a colour of Reaction

5. Antioxidant activity of Fruit extract in comparison with BHT

The antioxidant efficiency of substances is estimated using number of methods. Currently popular antioxidant methods used for determination of antioxidant capacity are diphenyl picrylhydrazyl (DPPH), β-carotene linoleic acid, and ABTS radical cation decolorization assay. The fruit extract of *G. Sylvestre*was screened for antioxidant potential using standard protocol areas follows.

a. **Determination of DPPH free radical scavenging activity by IC_{50} method**

The standard protocol of DPPH assay [16] was followed with slight modifications. Solutions of different concentrations of Fruit and standard, BHT, (20, 40, 60,100 µg/ml) in methanol were prepared. The

concentration selected for fruit extractis 20,40, 60, 100,200,300, 400 µg/mL. To the test solution (1 ml), DPPH solution (0.1mM, 1 ml) in methanol was added. Total volume was made upto 4 ml using methanol. After 30 minutes incubation in the dark, absorbance was recorded at 515 nm. All samples were analyzed in triplicates.The percentage inhibition (IC_{50}) was calculated by the following formula:

$$\% \text{ Inhibition } = \frac{[A_C-(A_t- A_b)]}{A_C} \text{ X 100}$$

Where,A_C = absorbance of control,

A_t = absorbance of test solutions/standard,

A_b % Inhibition = = Absorbance of blank solution.

The IC_{50} values of Fruit extract and BHT was determined by plotting graph of % inhibition of DPPH radical by antioxidant versus concentration of test solutions of *G.Sylvestre* including standard BHT.

b. **Determination of antioxidant activity using β-carotene / linoleic acid stem.**

Antioxidant activity was measured using standard protocol [17] with slight modifications. To 3.34 mg of β-carotene in chloroform solution (1 ml), 40 mg linoleic acid and 400 mg Tween-20 were added. The chloroform was then removed at 40°C under vacuum using a rotary evaporator. The resulting mixture was diluted with 10 ml distilled water and was mixed well. The emulsion was further made up to 100 ml 0.01 M hydrogen peroxide. Aliquots (2 ml) of this emulsion were transferred into different test tubes containing 0.1 ml of Fruit extract in methanol. In this experiment BHT was used as standard. A control containing 0.2 ml methanol and 4 ml of the above emulsion was prepared. The test tubes were placed in water bath at 50°C. Absorbance of all the samples at 470 nm were taken at zero time and after every 15 mins till the colour of β-carotene disappeared in the controlled reaction. The blank was prepared as described above without β-carotene. All samples were analyzed in triplicates. The Absorbance of all prepared test solutions was recorded at 470nm and % Inhibition is shown in Table 1.

The % inhibition was determined by the following equation:

$$\% \text{ Inhibition } = \frac{(A_{A(90)} - A_{C(90)}) \text{ x 100}}{(A_{C(0)} - A_{C(90)})}$$

Where, $A_{A(105)}$ is the absorbance of antioxidants at 90 min.,$A_{C(90)}$ is the absorbance of control at 105 min, $A_{c(0)}$ is the absorbance of control at 0 min.

c. ABTS radical cation decolorisation assay

The ABTS assay [18] was used to evaluate the ability of extract scavenge the ABTS radical. ABTS radical cations ($ABTS^+$) were produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hr before use. The concentration of plant extracts used for this assay are 100 µg/ml and 500 µg/ml. Into 1 ml of test solutions,0.6 ml ABTS solution was added and the final volume was made upto 2 ml with methanol. The control was prepared by adding methanol (1.4 ml) to ABTS solution (0.6 ml) while blank was prepared in the identical manner as the test solution but without ABTS solution. The Absorbance of all prepared test solutions was recorded at 745 nm and % Inhibition is shown in Table 1. All samples were analyzed in triplicates and the percentage inhibition was calculated using the formula:

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$$\% \text{ Inhibition } = \frac{(Ac- At) \text{ X 100}}{Ac}$$

Where, A_C is the absorbance Control and
A_t is absorbance of test solutions/Standard.

d. Total Phenolic content

The total Phenolic content was determined by the reported method [19] using Folin-Ciocalteau reagent. A solution of the sampleof concentration 100 µg/mL in methanol was prepared. To 1 ml of this solution, 1 ml Folin-Ciocalteau reagent was added. After 5 min. 10 ml of Na_2CO_3 (7%) was added to the mixture. This solution was diluted to 25 mL using distilled water. After incubation for 90 min., at room temperature, the absorbance against reagent blank was measured at 750 nm as shown in Table 5. Total Phenolic content of the samples was expressed as mg gallic acid equivalent (GAE) / 1 g. All the samples were analysed in triplicates. The results are expressed as mean± Standard deviation.

III. RESULTS AND DISCUSSION

The combined water and water ethanol extract obtained yielded the crude extract as dark, brownish, viscous residue. It was observed that fruit yielded **15±0.25%** of extract (FE).Qualitative phytochemical analysis studies shows that fruit extract contains phytochemicals like Tannins, Fixed oil and Fats, Flavanoids, Terpenoids, Saponins, Carbohydrates, Glycosides and Phenolics (Table No. 1).Table 2 indicates the % Inhibition of Fruit extract of *G.Sylvestre* in beta carotene and ABTS radical Scavenging assay. The IC_{50} value forfruit extract is 62µg/ml while that of for standard BHT is 20 µg/ml. Total phenolic content of fruit extracts is80 mg GAE / g dry weight. The higher phenolic content reflects that fruit of *G.sylvestre* contains phenolic compounds which are in support with the results obtained in Antioxidant activity.Results obtained in this study revealed that fruit of *G.sylvestre*has considerable antioxidant potential and source of phytochemicals.

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Tables & Figures

Table No. 1:Phytochemical analysis of the aqueous extract of *Gymnema sylvestre* fruit

Sr. No.	Qualitative Phytochemical analysis of the Water Extract of *Gymnema sylvestre* fruit	
	Phytochemical	**Fruit Water Extract**
1	Tannins	+
2	Fixed oil and Fats	+
3	Flavanoids	+
4	Terpenoids	++
5	Saponins	+
6	Steroids	-
7	Carbohydrates	+
8	Glycosides	+
9	Anthraquinones	-
10	Phenolics	+++
11	Catechin	-
12	Quinone	-
13	Reducing Sugar	+

+ = indicates presence of phytochemicals
- = indicates absence of phytochemicals.
+++ = shows high concentration.
++ = shows moderate concentration

Table 2: Antioxidant activity of the fruit aqueous extract of *G. sylvestre.*

Sr. No.	Compound/ Extract	β-Carotene-Linoleic acid assay % Inhibition		ABTS assay % Inhibition	
		100 µg	500 µg	100 µg	500 µg
1.	BHT	**80.8 ±0.22**	-	**100 ±0.00**	-
2.	Fruit Extract	**92.05±0.32**	**93.42±0.15**	**57.83±0.16**	**89.18±0.26**

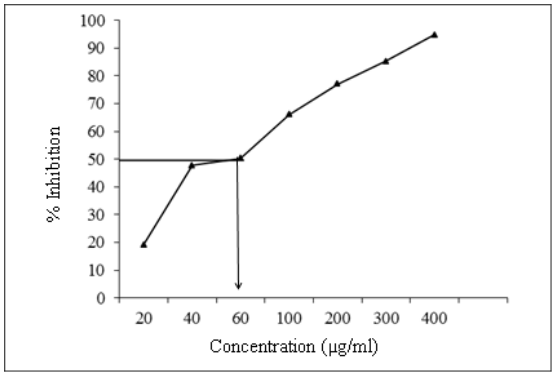

Fig.1 IC $_{50}$ value of fruit aqueous extract of *G. Sylvestre* extract

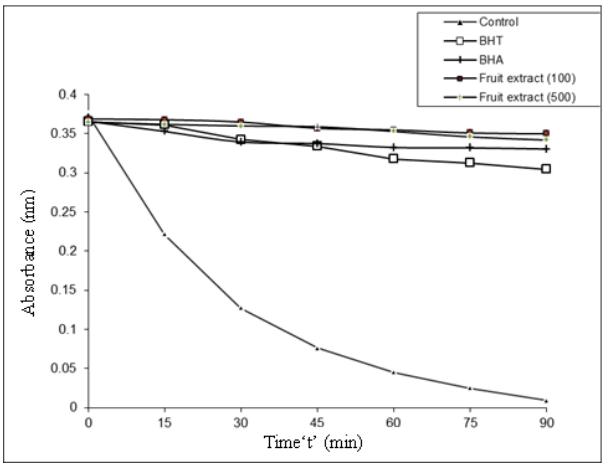

Fig. 2 Antioxidant activity of fruit aqueous extract of *G. Sylvestre* extract by Beta carotene bleaching method

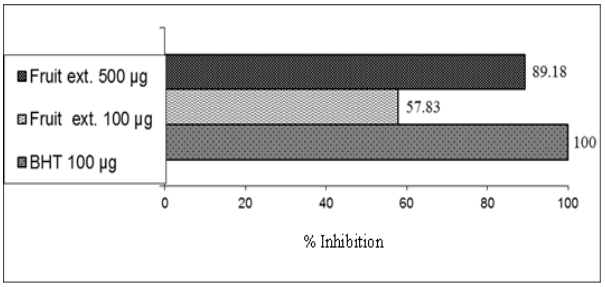

Fig. 3 Antioxidant activity of fruit aqueous extract of *G. Sylvestre* extract by ABTS radical Cation decolorization assay.