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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND IN VITRO EVALUATION OF ANTIOXIDANT & ANTIMICROBIAL ACTIVITY OF *GYMNEMA SYLVESTRE*

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ABSTRACT

The present study reports *in vitro* antioxidant and antimicrobial activities of leaf and stem extracts of *Gymnema sylvestre*. The water and water: ethanol (1:1) extracts of *Gymnema sylvestre* stem and leaves were screened for antioxidant and antimicrobial activity. The phytochemical analysis of the above extracts was also studied to check presence of active phytochemicals like Tannins, Flavonoids, Terpenoids, Saponins, Carbohydrates, Glycosides and Phenolics. Antioxidant activity of extracts was evaluated using butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) as standards. Antioxidant activity of stem extract was good in 2, 2-diphenylpicrylhydrazyl (DPPH), β-carotene bleaching and ABTS radical scavenging assays. Determination of the total phenolic content by Folin-Ciocalteau reagent indicated higher phenolic content in leaf extract.The antimicrobial activities of samples were investigated against eight bacterial strains. All the samples exhibited prominent activity against *Pr.mirabilis, B. subtilis* and *P. asplenii.* A moderate activity was indicated by the stem extract against *P. aeruginosa.*

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INTRODUCTION

Free radicals are responsible for oxidative damage known to cause various chronic diseases like atherosclerosis, Parkinson's diseases, Alzheimer's diseases, stroke, cancer chronic inflammatory, diseases, arthritis,and other degenerative diseases (Halli and Grootvele, 1987). Reactive Oxygen Species (ROS) are responsible for several disorders like aging, various inflammatory diseases, carcinogenesis neuro degenerative diseases and diabetes (Baynes and Thorpe, 1999). It is well known that diabetes is usually accompanied by increased production of ROS and impaired antioxidant defense (Narvez-mastache *et al*., 2007). ROS are usually scavenged by the antioxidants like glutathione peroxidase, catalase and superoxide dismutase which are naturally present in the body (Aruoma, 1994). In situation where endogenous antioxidant defenses are not totally efficient, antioxidant supplementation is required to diminish the oxidative damage.

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Available synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are associated with some side effects (Jafri *et al*., 2001; EI – Abhar *et al*., 2002). Consequently, there has been much attention in the antioxidant activity of naturally occurring substances (Hiruma – Lima *et al*., 2000). Many natural products (Soto C. Recoba *et al*., 2003) and medicinal plants possessing antioxidant properties are known to reduce oxidative stress. Extracts from plants namely *Albiziaamara, Achyranthes aspera, Cassia fistula, Cassia auriculata* and *Datura stramonium* possess antioxidant activity (Kumar *et al*., 2008). Extract of leaves and stem of *Raphanus sativus* L. possess radical scavenging activity (Beevi *et al*., 2010). *Gymnema sylvestre* R.Br. (Family: Asclepiadaceace), commonly known as 'Gurmar', is a well-known indigenous medicinal plant used in the treatment of diabetes and many other ailments. The plant is woody climber, located in central and western India, Tropical Africa and Australia. A recent review describes the antimicrobial, hepatoprotective, antihypercholesterolemic and anti-inflammatory activities of leaves of this plant and states that they are used for making antidiabetic formulations in folk, ayurvedic and homeopathic medicines (Kanetkar *et al*., 2007).

The recent review articles show that *Gymnema sylvestre* R.Br, used to control diabetes, obesity, atherosclerosis etc., by traditional medicinal practitioners of India (Subramaniyan Vijayakumar and Srinivasan Prabhu, 2014; Surendra Kumar *et al*., 2015; Lalit Kishore *et al*., 2014). The leaves have a unique property of inhibiting the ability to taste sweet substances (Maeda *et al*., 1989). Leaves are also used in the treatment of bronchitis, jaundice and asthama (Nakamura *et al*., 1989). Formulations of the active constituents from leaves were also useful against obesity (Yoshikawa *et al*., 1993). Due to a range of biological activities, considerable work has been done to isolate and identify chemical constituents of these leaves. It is well known that a group of more than twenty saponin glycosides of olenane-type including mixture of gymnemic acids I-XVIII (antisweet compounds) and gymnema saponins are the active constituents of these leaves (Agarwal *et al*., 2000). Gymnemic acid is present in different parts of the plant like root, flowers, internodes, seeds and stalks, however leaves of *G. Sylvestre* are the major source of gymnemic acids (Deokule and Pokharkar, 2009). Besides these, other constituents present in the leaves include lupeol, stigmasterol, flavones, anthraquinones, phytin, inositol, tartaric acid, Choline, β-amyrinn, resin, α and β Chlorophyll, betain, alkaloids, triethylamine and d-quercitol (Tiwari *et al*., 2014).

There are reports of aqueous leaf extract of *G. sylvestr*e displaying lavicidal effect on *Culexqinquifaciatus* mosquito larvae (Khanna and Kannabiran, 2007). It is evident from the available literature that some of the antidiabetic plants possess antioxidant activity. Antioxidant property of alcoholic leaf extract (Kang *et al*., 2012) and antibiotic activity of *G. sylvestre* extracts (Saumendu *et al*., 2010) was reported. Also, there is limited number of reports on antimicrobial activities of *G. sylvestre* (Satdive *et al*., 2003; Khanna and Kannabiran, 2008). Literature survey indicates that the antioxidant and antimicrobial properties of the *G. sylvestre* stem is hitherto unknown. A study was therefore undertaken to evaluate and compare the antioxidant and antimicrobial activity of leaf extract (LE) and stem extract (SE). In our ongoing programme on developing bioactive extracts, antioxidant properties of *Gymnema sylvestre* leaf essential oil were recently been demonstrated (Naik *et al*., 2011).

MATERIALS AND METHODS

Plant material

The plants of *G. sylvestre* (2 Kg) 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).

Chemicals

Butylated hydroxy anisole (BHA), Butylated hydroxy toluene (BHT) and Tween-20 were purchased from Loba Chemicals, linoleic acid was purchased from SRL and β-carotene from HIMEDIA, Folin-Ciocalteu reagent was purchased from Qualigens. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2, 2-azinobis-(-3-ethyl benzothiazoline-6-sulphonic acid) diammonium salt (ABTS) and ammonium persulphate were procured from Fluka, USA. All the solvents used were of analytical grade.

Cultures of Microorganisms

Pseudomonas aeruginosa (Gram *–ve,* ARICHM-1), *Bacillus subtilis* (Gram *+ve,* ARICHM-2), *Bacillus cereus* (Gram *+ve,*ARICHM-3), *Escherichia coli* (Gram *–ve,* ARICHM-4), *Staphylococcus aureus* (Gram *–ve,* ARICHM-5), *Proteus mirabilis* (Gram *–ve,* ARICHM-6), *Pseudomonas asplenii* (Gram *–ve,* ARICHM-7) and *Candida albicans* (ARICHM-8) were obtained from the culture collection of Agharkar Research Institute.

Preparation of extracts from G. Sylvestre

Dried and powdered leaves and stem (500 g) of *G.sylvestre* were subjected to cold extraction with n-hexane (1.5 lit) at room temperature (4 x 16 h). The dried powder was then extracted with distilled water (1.5 lit) at room temperature (3 x 6 h). The combined water extract was concentrated under reduced pressure at 60°C. The extraction was then carried out using water: ethanol (1:1) at room temperature. The combined extracts of leaf and stem were concentrated under reduced pressure using rotary vacuum evaporator.

Phytochemical Screnning

The phytochemical Screening of the extracts was done using standard procedure as described in (27, 28, 29, 30). The following qualitative tests were carried out as follows.

1.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 addedto a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2hrs. Formation of soap or partial neutralization of alkaliindicates the presence of fixed oil and fats.

2.Steroids: 10 mg of the each 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.

3.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.

4.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.

5.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

6.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.

7.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.

8.Anthraquinones: To the extract Magnesium acetate solution was added the pink colour developed indicates the presence of Anthraquinones.

9.Reducing Sugar: Aqueous extract was added to boiling Fehling's solution (A and B). The solution was observed for a colour of Reaction.

10.Glycoside test: Few mg of extract was taken in a dried test tube and dissolved in 2 ml of methanol. 1 ml alpha naphtolalcoholic solution was added from the sides of the test tube.After adding 1 ml alpha naphtolalcoholic solution from side of the test tube, bluish ring was developed which indicated that the presence of glycosides

Antioxidant assay

Determination of free radical scavenging activity (DPPH)

7 The standard protocol of DPPH assay (Mokbel and Hashinaga, 2005) was followed with slight modifications. Solutions of different concentrations of samples with standard, BHT, (20, 40, 60,100 µg/ml) in methanol were prepared. 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. The percentage of inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{[A_0-(A_t-}{A_0} \quad X\ 100$$

Where,
A_0 = absorbance of control,
A_t= absorbance of test solutions/standard,
A_b = absorbance of blank solution.

Antioxidant activity of the samples is expressed as IC_{50} values. The IC_{50} value is defined as the concentration of sample which inhibits 50 % of DPPH radical. All the experiments were performed in triplicate.

Determination of antioxidant activity using Beta-carotene bleaching assay

Antioxidant activity was measured using standard protocol (Pellegrini *et al*., 1999) with slight modifications. To 3.34 mg of Beta-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 with 0.01M hydrogen peroxide. The test solution of different concentrations (1 mg/ml and 5 mg/ml) of each sample and the standard solutions of BHA and BHT (1 mg/ml) in methanol were prepared. Aliquots (2 ml) of emulsion were transferred into different test tubes containing 0.1 ml of test samples and standardsin methanol. In this experiment BHA and BHT were used as standards. 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 control. The blank was prepared as described above without β-carotene. The % inhibition was determined by the following equation:

$$\% \text{ Inhibition} = \frac{(A_{A(105)} - A_{C(105)})\ \text{x } 100}{(A_{C(0)} - A_{C(105)})}$$

Where, $A_{A(105)}$ is the absorbance of antioxidants at 105 min., $A_{C(105)}$ is the absorbance of control at 105 min., $A_{c(0)}$ is the absorbance of control at 0 min. All the experiments were performed in triplicate.

ABTS radical cation decolorisation assay

Pellegini's procedure (33) was used to evaluate the ability of extracts to scavenge the $ABTS^{\cdot+}$ radical. $ABTS^{\cdot+}$ radical cations were generated by reacting ABTS solution (7 mM, 3 ml) with ammonium persulphate (2.45 mM, 15 ml). The reaction mixture was allowed to stand at room temperature for 16 h before use. The test solutions (100 µg /ml and 500 µg /ml) of each sample and the standard solutions of BHA and BHT (100 µg /ml) in methanol were prepared. The ABTS solution (0.6 ml) was added to each test tube containing test solution and standards (1 ml each) and the final volume was made upto 2 ml. 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. Absorbance was read at 745 nm. All the samples were analyzed in triplicate.

$$\% \text{ Inhibition} = \frac{(\text{Control} - \text{Test}) \text{ X } 100}{\text{Control}}$$

Total Phenolic content

The total phenolic content was determined by the reported method (34) using Folin-Ciocalteau reagent. A solution of the sample of 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 determined at 750 nm. Total Phenolic content of the samples were expressed as mg gallic acid equivalent (GAE) / 1 g. All the experiments were performed in triplicates.

Antimicrobial Activity

Antimicrobial activity was examined by agar well-diffusion method (32) using microorganism ÷ Pure cultures of bacteria, *Bacillus subtilis* (Gram +*ve,* ARICHM-2), *Bacillus cereus*

(Gram +*ve,* ARICHM-3) *and Pseudomonas aeruginosa* (Gram –*ve,* ARICHM-1), *Escherichia coli* (Gram –*ve,* ARICHM-4), *Staphylococcus aureus* (Gram –*ve,* ARICHM-5), *Proteus mirabilis* (Gm –*ve,* ARICHM-6), *Pseudomonas asplenii* (Gram –*ve,* ARICHM-7). The culture of fungus *Candida albicans* (ARICHM-8) obtained from the culture collection of ARI. The mother cultures of each micro-organism were allowed to stand for 24 h in order to reach the stationary phase of growth before the assays. Petri dishes containing the mother cultures with proper sterile media (MH Agar medium) were used for bacteria. The media were inoculated to obtain the micro-organism concentration of 130 x 10^7 colony forming units per ml (cfu / ml). The wells were made by sterile cock borer (6 mm dia.). Each well was loaded with 40 µl sample (30 mg / ml). All the plates were kept at 5^0C for half an hour for diffusion. The plates were then incubated for 24 h at 37^0C and the diameters of growth inhibition zones were measured using methanol as a blank. Each assay was performed in triplicates on three independent experimental runs. The minimum inhibitory concentration (MIC) of extracts indicating clear inhibition was determined by well diffusion method (32).

Statistical analysis

All the statistical analyses were performed using SPSS version 11.0. Values are presented as a means ± standard deviation. One way Analysis of variance was carried out and differences between variables were tested for significance by post hoc Tukey's HSD multiple comparison test. Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The extraction of the defatted leaves with water & Water: Ethanol (1:1) yielded 160 g (32%) of the residue as a dark viscous semi solid. Similarly the extraction of stem yielded 115 g (23%) of the residue. The results are presented in Table 1.

Phytochemical Screnning

The research work was carried out on *G. Sylvestre* medicinal plant which shows that presence or absence of active phytochemicals like Tannins, Flavanoids, Terpenoids, Saponins, Carbohydrates, Glycosides, Reducing sugars, anthraquinones, Anthocyanins and Phenolics. The results were summarized in Table 2. In our studies the Plant *G.sylvestre* contains Fixed oils & fats, Tannins, Flavanoids, Terpenoids, Saponins, Carbohydrates, Glycosides and Phenolics. The other phytochemicals steroids, anthraquinones and Anthocyanins were absent.

DPPH radical scavenging activity

The free radical scavenging capacities of leaf and stem extracts was determined by DPPH assay. The results are shown in Figure 1and Table 1. Known antioxidants, BHT and BHA, were used to validate this assay. Relatively stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The IC_{50} value for leaf extract and stem extract was found to be 111.1µg /ml and 122.1 µg /ml respectively in comparison to that of standard BHT (20 µg/ml) (Figure 1, Table 1), this indicates the significant antioxidant activity of Plant extract. It is noteworthy that the observed antioxidant activity of leaf extract is superior to that of the stem extract of *G. sylvestre*.

Table 1. Yield (%), Phenolic Content and IC_{50} values of samples of *G. Sylvestre*

S.No.	Compound/Extract	Leaf extract	Stem Extract	BHT
1.	Yield (%)	32%	23%	-
2	IC_{50} (µg/ml) as determined by DPPH assay	111.4	122.1	20
3.	Phenolic content (mg/GAE dry weight)	11 ± 2.20	13 ±1.20	-

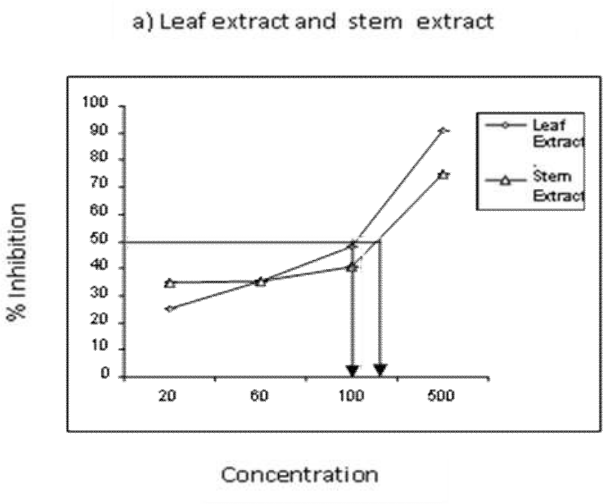

Fig.1. DPPH free radical-scavenging activity of Leaf and Stem Extractof *G. Sylvestre*

Beta- carotene bleaching assay

Lipid peroxidation in food products leads to off-flavor and other quality like colour and texture are also affected. To prevent this, food industry uses various additives with antioxidant activities. Results of the assay are presented in Figure 3 and Table 2. The percent inhibition was calculated after 90 minutes. In linoleic acid- β - carotene bleaching method, oxidation of linoleic acid was significantly inhibited by leaf & stem extract at both the concentrations, i.e. 100µg/ml and 500µg/ml. In this assay the stem extract showed stronger activity than that of leaf extract at 100µg/ml and 500µg/ml. The control showed decrease in the absorbance with time due to the formation of peroxides in the absence of antioxidants (Figure 3). Stem extract showed moderate activity (67.6 ± 0.41%) at 100 µg activity of stem extract and LGA did not increase much at 500 µg concentration, (78.4 ± 0.33% inhibition) (Figure 2, Table 3).

Table 2. Phytochemical Screnning of *G.sylvestre*

S.No.	Phytochemicals	Leaves	Stem
1	Tannins	+	+
2	Fixed oils and fats	+++	+++
3	Flavanoids	++	++
4	Terpenoids	+	+
5	Saponins	+++	+++
6	Steroids	-	-
7	Glycosides	++	++
8	Anthraquinones	-	-
9	Anthocyanins	-	-
10	Phenolics	++	++

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

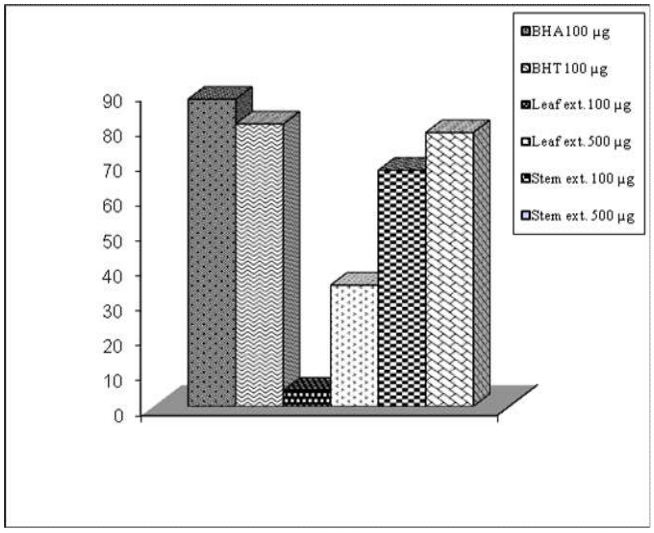

Fig. 2. Antioxidant activity by β-carotene method

Table 3. Antioxidant activity of the extract from *G. sylvestre*

S. 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.	BHA	88±0.06	-	100±0.00	-
3.	Stem extract	67.6±0.42	78.4±0.36	17.09±0.14	65.9±0.00
5.	Leaf extract	4.6±0.335	34.7±0.30	12.89±0.09	32.11±0.69

Values are expressed as means ± Standard deviations (n=3)
*Concentration in reaction mixture
** % Inhibition after 90 min

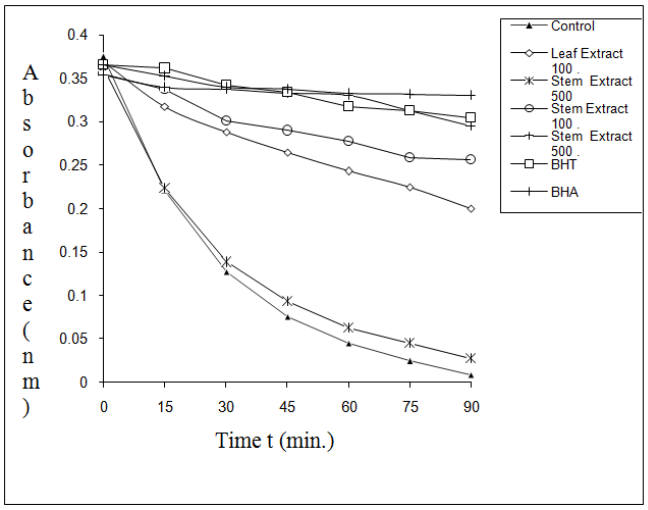

Fig.3. Variation of antioxidant activity of *G. sylvestre* Leaf and stem extract

ABTS radical cation decolorization assay

This assay is based on the inhibition of the absorbance of radical cation, $ABTS^{\cdot+}$ which has a characteristic long wavelength absorption spectrum. The antioxidant activity of extracts and of leaf and stem were examined using $ABTS^{\cdot+}$ radical cation decolorisation assay. The inhibition by the test samples in the ABTS assay is shown in Figure 4 and Table 3. BHT and BHA were used as standards to validate this assay. As depicted in the above two assays, the activities shown by stem and leaf extracts were low at 100 µg concentration ($p<0.01$). At 500 µg concentration, stem extract and leaf extract showed moderate activities i.e. 65.9 ± 0.13% and 32.11 ± 0.24% respectively.

Total phenolic contents

Total phenolic content of stem and leaf extract was very low (13mg GAE/g and 11mg GAE /g dry weight respectively). The presence of antioxidants in the *G. sylvestre* plant is indicated by the inhibition shown in various antioxidant assays. The major bioactive constituents of *Gymnema sylvestre* contains group of olenane type triperpenoid saponins known as gymnemic acids. The triperpenoid obtained from *Salvia macrochlamy* shows antioxidantactivity (Mokbel and Hashinaga, 2005) which supports strong antioxidant activity of triperpenoids present in leaves of *G. sylvestre.* This needs further investigation. DPPH and ABTS radical scavenging activity depicted by leaf extract may be correlated to its highest phenolic content, which indicates its potency to form a chemical basis of various applications in pharmaceuticals as well as food industries.

Table 4. Antimicrobial activity of extract from *G. sylvestre* against various microbial strains

Microbial strains	Leaf extract	Stem extract	Gentamycin (10µg)
Pseudomonas aeruginosa	-	10	18
Pseudomonas asplenii	13	12	20
Esherichia coli	-	11	20
Bacillus subtilis	14	12	25
Proteus mirabilis	11	13	25
Staphylococcus aureus	-	-	-
Bacillus cereus	-	-	-
Candida albicans	-	-	11

- indicates no zone of inhibition,
Zone of Inhibition are in mm.

Table 5. Minimum Inhibitory Concentration (MIC) of extracts from *G. sylvestre* plant against various microbial strains

Sample	Leaf extract (mg/ml)	Stem extract (mg/ml)
Pseudomonas aeruginosa	-	50
Pseudomonas asplenii	30	30
Esherichia coli	-	-
Bacillus subtilis	30	30
Proteus mirabilis	30	30
Candida albicans	-	50

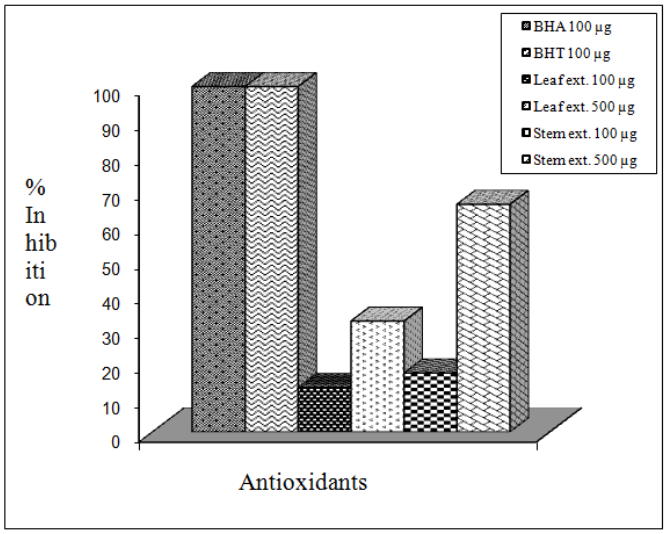

Fig.4. Antioxidant activity of *G. Sylvestre* by ABTS method

Antibacterial activity

Phenolics, Saponins and flavonoids are well known phytochemicals which exhibit antioxidant, antidiabetic, anti-inflammatory, anti-cancer and other biological activities. The water extract of *G. sylvestre* contains active compounds like gymnemic acids I-XVIII and gymnema saponins. Table 3 shows the antibacterial activities exhibited by aqueous extracts was better to that of earlier reported activity of ethanolic leaf extract (Satdive *et al*., 2003) but lower than that of saponin fractions (Khanna and Kannabiran, 2008) of *G. sylvestre* leaves. The leaf and stem extracts showed moderate antibacterial activity (Table 4). Among the test samples, stem extract shows antimicrobial activity against bacterial strains with greater zone of inhibition including E-*coli* which is resistant to external agents like hydrophilic dyes, antibiotics and detergents due to lypopolysaccharides in their outer membrane (Khaled *et al*., 2009). In line to our findings, other previous studies have demonstrated In vitro antibacterial activities against E. coli, *Staphylococcus aureus*, *Pseudomonas aeruginos*a (Lilly *et al*., 2014). The MIC of leaf and stem extract is summarized in Table 5. In the previous study, the ethanolic extracts of saponinsfractionsof *G. Sylvestre* leaves were found to possess antimicrobial activity. However there are no reports on antimicrobial activity of stem extract.

Abbrevations

BHA: Butylated hydroxy anisole,
BHT: Butylated hydroxy toluene,
DPPH: 2, 2-Diphenyl-1-picrylhydrazyl,
ABTS: 2, 2-azinobis-(-3-ethyl benzothiazoline-6-sulphonic acid) diamonium salt.

Conclusion

The leaves of *G. sylvestre* are well known for a very long time as an antidiabetic drug in the traditional system of Ayurvedic Medicine. The diabetes is usually accompanied by increased production of reactive oxygen species (ROS) and impaired oxidative defense. Several studies using in vitro models of diabetes have demonstrated that plant extracts decreased oxidative stress and enhanced the activities of components of the endogenous antioxidant system. Plant based phenols are known to exhibit antioxidant activity through a variety of mechanism including scavenging of ROS and inhibiting lipid peroxidation. In our present study, we have demonstrated that the antioxidant activity of stem extract is superior than leaf extract. We therefore conclude that the potential of the stem extract as an antidiabetic drug is much more effective than traditionally used leaf extract of gymnema plant.

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