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Original Article

Phytochemical Analysis and Antioxidant Activities of *Gymnema sylvestre* R. Br. Leaf Extracts

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ABSTRACT

Objective: The present study aims to determine the antioxidant properties and phytochemical screening of *Gymnema sylvestre* plant. **Materials and Methods:** The qualitative and quantitative analyses were carried out for the methanol extract using standard procedures. The antioxidant activities were carried out by DPPH free radical, OH^- radical, H_2O_2 radical, O_2^- radical and phosphomolybdenum reduction assay. **Results:** The leaves of *G. sylvestre* methanol extract showed good radical scavenging as well as reducing power activities. The total phenolic and flavonoid content were 19.87 ± 0.32 mg/g and 2.65 ± 0.12 mg/g respectively. **Conclusion:** The methanol extract of leaves of *G. sylvestre* possesses significant antioxidant activities.

Key words: Antioxidant, Total phenolic, Flavonoid, *Gymnema sylvestre*.

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INTRODUCTION

Antioxidants are fundamental substances that possess the ability to protect the body from damage caused by free radical-induced oxidative stress.[1] Free radicals are molecules containing one or more unpaired electrons in atomic or molecular orbitals.[2] There is increasing evidence that abnormal production of free radicals leads to increased oxidative stress on cellular structure and causes changes in molecular pathways that underpin the pathogenesis of several important diseases, cancer and in the process of physiological aging.[3] Diabetes is one of the major endocrine disorders affecting major systems of the body, leading to multiorgan complications.[4] Verbal hypoglycaemic agents like sulphonylureas and biguanides are the predictable drugs used for the treatment but the undesirable side effect associated with these drugs is a major drawback.[5] The herbal medicines are becoming accepted due to better results and safe use as compared to marketed drugs and more effective treatment of health problem.[6]

G. *sylvestre* is an indigenous medicinal herb belonging to the class dicotyledonous of the family Asclepiadaceae. The plant is a good quality of many bioactive compounds.[7] It has deep roots in history, being one of the major botanical used in Ayurvedic system of medicine to treat conditions ranging from diabetes, malaria, to snake bite.[8] The aim of present study was to determine the phytochemical screening, antioxidant activity and free radical scavenging activity of the methanol extract of *G. sylvestre*.

MATERIALS AND METHODS

Collection of plant material

The plant material of *G. sylvestre* was collected in the month of December to January 2018 from Kandhamal district, Odisha, India. The plant was identified and deposited in the Herbarium house; Department of Botany, Berhampur University, Odisha, India and confirmed by the specialists. The selected plant part was removed and then washed under running tap water to remove dirt. The plant sample was then oven dried at 60°C for few days and was crushed into powder and stored in polythene bags for future use.

Preparation of Plant extract

Measured plant part was extracted with methanol by using soxhlet apparatus method. Finally, the methanol extract was evaporated in a rotary evaporator to obtain the respective extract and stored in a freeze condition at -20°C until used for future analysis.[9] Percent of yield[10] was calculated as follows;

$$\text{Extract yield } \% = W_2 - W_1/W_0 \times 100$$

Where, W_2= the weight of the extract and the container, W_1= the weight of the container alone and W_0= the weight of the initial dried sample.

Qualitative analysis of Phytochemicals

The qualitative analysis of crude extract for their bioactive ingredients was carried out by following standard procedures.[11]

Quantitative analysis of Phytochemicals

Estimation of Total Phenolic Content (TPC)

The concentration of phenolic in plant extract was determined by the Folin- Ciocalteu method with little modification.[12] 20 µl of methanol extract (1mg/ml) into a test tube and add 1.58 ml of distilled water then add 7% of 100 µl of Folin- Ciocalteu's reagent. Wait for 8 mins, then 10% of 300 µl of saturated sodium

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carbonate solution (250 g/l) was added. Incubated the solution for 2 hr at room temperature, the absorbance was measured at 765 nm in triplicate. Gallic acid (10-500 mg/l) was used for calibration of the standard curve. The results were expressed as milligram gallic acid equivalent (mg GAE/g) dry weight of plant material.

Estimation of Total Flavonoid Content (TFC)

The total flavonoid content was estimated by using Spectrophotometric method.[13] The crude extract contained 1 ml of the methanol solution of the extract in 1mg/ml concentration and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The solutions were incubated for an hour at room temperature. The absorbance was obtained at 415 nm in triplicate. The same protocol was repeated for the standard solution of rutin and the standard curve was constructed. Then, the content of flavonoid in the extract was expressed in terms of rutin equivalent (mg of RUE/ g) dry weight of plant material.

In vitro antioxidant assays

Phosphomolybdenum assay

The total antioxidant capacity of the methanol extract was determined by the phosphomolybdenum method.[14] The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate complex at acid pH. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 90°C for 90 mins. Then, after cooling the absorbance of the solution was estimated at 695 nm using a spectrophotometer against the blank. Methanol (0.3 mL) in the place of the extract was used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid (10-500 µg/mL) with methanol.

DPPH radicals scavenging activity

The free radical scavenging capacity of the methanol extracts was determined by using DPPH assay.[15] DPPH solution (0.004%, w/v) was prepared in methanol. Stock solution (1 mg/ml) of methanol extract of plant and standard ascorbic acid (0.05 g/ml) were prepared using methanol. Various concentrations (10-500 µg/ml) of the plant extract and ascorbic acid were taken in a test tube and 1ml freshly prepared DPPH solution was added, the test tubes were protected from light by covering with aluminium foil. The final volume in each test tube was made to 2 ml with methanol and incubated in dark for 30 mins at room temperature. After incubation the absorbance was read at 517 nm using a spectrophotometer. The control sample was prepared to contain the same volume of methanol and DPPH without any extract and reference ascorbic acid. Methanol was served as blank. The radical scavenging was calculated by the following formula;

$$\% \text{ Inhibition} = [(\text{Abs of control} - \text{Abs of sample})/ \text{Abs of control}] \times 100$$

Hydroxyl radical scavenging activity

The reaction mixture (3 ml) containing 1 ml $FeSO_4$ (1.5 mM), 0.7 mL hydrogen peroxide (6 mM), 10% of 0.3 ml sodium salicylate (20 mM) and varying concentrations of the extracts (10-500µg/ml) were taken. After incubation for 1hr at 37°C, the absorbance of the hydroxylated salicylated complex was measured at 562 nm.[16] The ascorbic acid was used as the standard. The percentage scavenging effect was calculated as:

% scavenging activity = [1- (A1 – A2)/ A0] × 100, where A0 was the absorbance of the control (without extract), A1 was the absorbance in the presence of the extract with sodium salicylate and A2 was the absorbance without sodium salicylate.

Superoxide anion radical scavenging activity

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) under aerobic condition.[17] Tris HCl buffer (3ml, 16 mM, pH 8.0) containing 1 ml NBT (50 µM) solution, 1 ml NADH (78 µM) solution and a sample solution of extract (10-500µg/ml) in distilled water mixed. The reaction was started when 1 ml of PMS solution (10 µM) was added to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance was read at 560 nm against the corresponding blank samples. Ascorbic acid was used as a standard. The decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

Hydrogen peroxide radical scavenging activity

The capability of the extract to scavenge hydrogen peroxide (H_2O_2) was estimated according to the method of Nabavi *et al*.[18] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer, pH 7.4. The concentration of hydrogen peroxide was determined by absorption at 230 nm using a UV- visible spectrophotometer. The extracts (10-500µg/ml) in distilled water were added to a hydrogen peroxide solution at 230 nm was determined after 10 mins against the blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a standard.

Statistical Analysis

All experimental measurements were carried out in triplicate and are expressed as an average of three analysis ± standard deviation (SD).

RESULTS

The percentage of yield of methanol extract of *G. sylvestre* was found to be 35.07% w/w.

Qualitative analysis of Phytochemicals

The qualitative phytochemical screening conducted on the methanol extract of *G. sylvestre* showed the presence of a wide class of bioactive compounds, including alkaloid, terpenoids, phenols, tannins, reducing sugar, protein, steroid, anthocyanin, coumarin, leucoanthocyanin and glycosides. Saponin was absent in methanol extract of *G. sylvestre* (Table 1).

Quantitative analysis of Phytochemicals

The total phenolic content of methanol extract of *G. sylvestre* measured by Folin- ciocalteu reagent in terms of gallic acid equivalent (the standard curve equation: $y = 0.0006x + 0.0367$, $R^2 = 0.9317$). The value obtained for the concentration of total phenols is 19.87 mg/g dry weight. The flavonoid content was expressed in terms of rutin equivalent (the standard curve equation: $y = 0.0013 x + 0.0021$, $R^2 = 0.9877$). The concentration of flavonoid in plant extract is 2.65 mg/g dry weight (Table 2). The phenolic compounds from plants are known to be good natural antioxidants.[2] Phenolic and flavonoid compounds of the plant which carry regarding substantial antioxidant activity and several biological activities including; anthelmintic, analgesic, anti-inflammatory, antimicrobial and anti-allergic properties.[19]

In vitro antioxidant assays

Phosphomolybdenum assay

The total antioxidant potential of the methanol extract of *G. sylvestre* was presented as ascorbic acid equivalents (AAE) per gram. The plant extract exhibited a decline of Mo (VI) to Mo (V). The highest total antioxidant capacity was observed for AAE with a value of 164.15 ± 0.14 mg AAE/g. The plant extract showed least antioxidant capacity (132.53 ± 0.03) (Table 2).

DPPH radicals scavenging activity

DPPH radical scavenging activity of methanol extract of *G. sylvestre* was compared with Ascorbic acid. In this assay, the plant extract showed the most powerful free radical scavenging activity with value ranges from 27.119 to 77.966% at the concentration from 10-500µg/ml and the standard ranges from 71.186 to 94.915% (Figure 1).

Hydroxyl radical assay

The methanol extract of *G. sylvestre* showed a significant dose-depended hydroxyl radical scavenging activity and it reached up to 73.096% at the concentration of 500µg/ml. However, the ascorbic acid which was used as a standard showed better radical scavenging effect 98.477% at the concentration of 500µg/ml (Figure 2).

Superoxide radical assay

The methanol extract of *G. sylvestre* scavenges the superoxide radicals up to 56.250% at 500µg/ml concentration, whereas standard ascorbic acid at the same concentration scavenged 71.875%. The abilities of the plant extract and ascorbic acid to quench superoxide radicals from the reaction mixture is reflected in the decrease of the absorbance (Figure 3).

Table 1: Qualitative phytochemical analysis of methanol extract of *G. sylvestre*.

Phytochemicals	Methanol
Alkaloids	+
Terpenoids	+
Phenols and Tannins	+
Reducing sugar	+
Saponin	-
Protein	+
Steroid	+
Anthocyanin	+
Coumarin	+
Leucoanthocyanin	+
Glycosides	+

+ = Present; - = Absent

Table 2: Phytochemicals and antioxidant power of Methanol extract of *G. sylvestre*.

Phytochemicals	Value
Total phenolic content (mg of GAE/ g)	19.87 ± 0.32
Total flavonoid content (mg of RUE/g)	2. 65 ± 0.12
Total antioxidant activity (mg of AAE/g)	132.53 ± 0.03

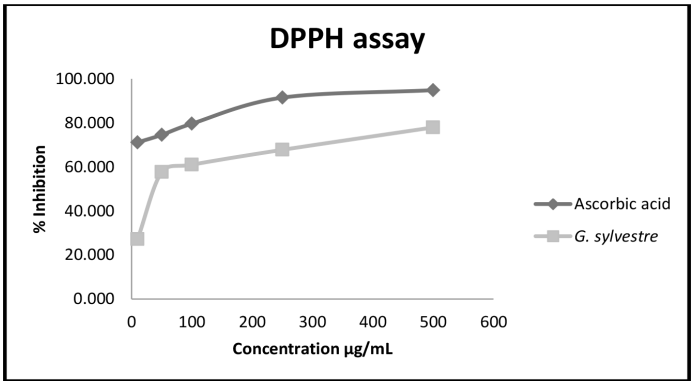

Figure 1: DPPH scavenging of methanol extract of *G. sylvestre* compared to that Ascorbic acid (AA).

Hydrogen peroxide radical assay

The free radical scavenging activity of *G. sylvestre* was determined by hydrogen peroxide (H_2O_2) scavenging method. From the results, the plant extract showed concentration-dependent activity and the hydrogen peroxide scavenging effect was 72.375% at a concentration of 500µg/ml. This was comparable to the scavenging effect of ascorbic acid (88.540%) (Figure 4).

DISCUSSION

The antioxidant activity of polyphenolic compounds present in *G. sylvestre* has been demonstrated earlier.[20] However, the ability of methanol extract of *G. sylvestre* to scavenge free radicals in chemical and biological systems has not been comprehensively investigated.

This study was made to determine the total phenolic content, flavonoid content, as well as antioxidant activity. Phenolics are made of one or more aromatic rings bearing one or more hydroxyl groups and are therefore potentially able to reduce free radicals by forming stabilized phenoxyl radicals.[21] Recent investigations have demonstrated that polyphenols and flavonoids contribute in a great way to the antioxidant activity of medicinal plants.[22]

Antioxidant activity of medicinal plant is the most efficient way of combating tissue injuries undesired transformations and preventing health risks.[23] The total antioxidant activity of methanol plant extract may serve as a significant indicator of its potential antioxidant activity.

The antioxidant activity of plant extract was also discernible in the DPPH radical assay, which primary evaluates proton radical scavenging

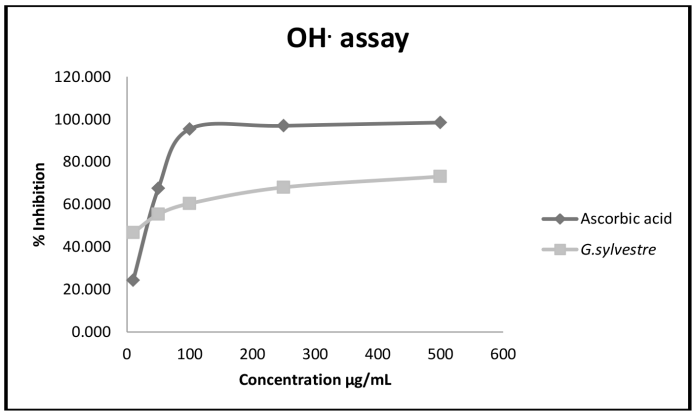

Figure 2: Hydroxyl radical scavenging of methanol extract of *G. sylvestre* compared to that Ascorbic acid (AA).

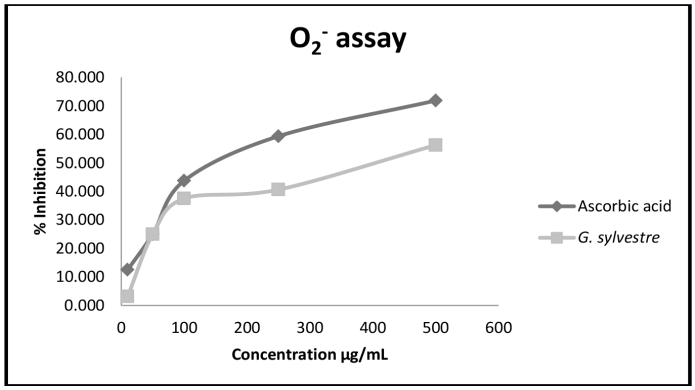

Figure 3: Superoxide radical scavenging of methanol extract of *G. sylvestre* compared to that Ascorbic acid (AA).

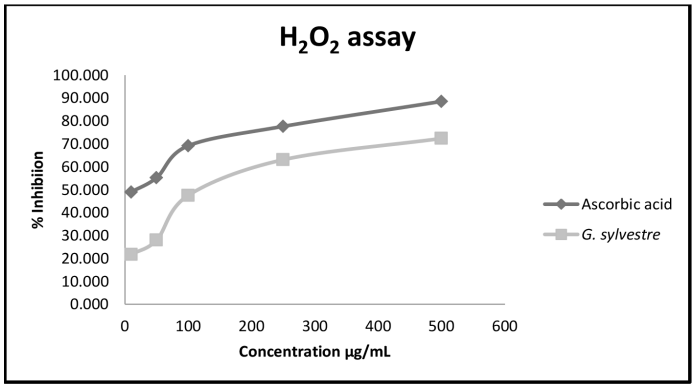

Figure 4: Hydrogen peroxide radical scavenging of methanol extract of *G. sylvestre* compared to that Ascorbic acid (AA).

ability. DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure of proton radical scavengers.[24] In the present study, the plant extract showed a concentration-dependent scavenging of DPPH radical, which may be attributable to its hydrogen donating ability.

Hydrogen peroxide itself is not very reactive, but occasionally it is toxic to cells because it may give rise to hydroxyl radical. Thus, removing hydrogen peroxide is extremely vital for antioxidant defense in cell system.[25] Hydroxyl radical scavenging ability of *G. sylvestre* extract is directly related to its antioxidant activity.

Superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA.[26] The superoxide scavenging activity of methanol extract of *G. sylvestre* has the potential to scavenge superoxide anions. It was reported that the superoxide anion scavenging activity could be due to the action of a free hydroxyl group of phenolic compounds.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play important role in absorbing and neutralizing free radicals.[27] Flavonoids and tannins seem to be a most promising polyphenolic compound.[28] This result suggests that the methanol extract of *G. sylvestre* showed higher antioxidant activity due to the presence of phenols, tannins and high amount of flavonoids.

CONCLUSION

In the present study, various *in vitro* assays were carried out to evaluate the antioxidant activity of methanol extract of *G. sylvestre*. The plan plant extract was found to possess good antioxidant activity which may be credited to the presence of phytochemicals like alkaloids, steroid, protein, terpenoids, glycosides, etc. Further studies are required to substitute of plant extract as a natural drug for the treatment of diseases caused by free radicals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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