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ORIGINAL RESEARCH

In vitro and *in vivo* antioxidant activities of the hydro-alcoholic extract of *Stemona tuberosa* Lour.

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The study was undertaken to evaluate the in vitro and in vivo antioxidant activities of a traditionally used medicinal plant, *Stemona tuberosa*. The roots of the *S. tuberosa* were processed for chemical extraction with a hydro-alcoholic solution. Phytochemical screening was done to evaluate the secondary metabolite constituents. In vitro antioxidant activity was assessed by different methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, hydroxyl radical scavenging method and reducing power capacity. The total phenolic content and total flavonoid content were also determined. In vivo antioxidant activity was performed on 36 male rats, randomly divided into six groups, and then treated with different antioxidant drugs and the plant extract. Oxidative stress was induced in Group 2 to Group 6 animals with streptozotocin (STZ). On the 15th day, the animals were sacrificed, and biochemical analysis was done to estimate the antioxidant parameters. The hydro-alcoholic extracts of the plant contained alkaloids, carbohydrates, proteins and amino acids, steroids, triterpenoids, phenols, tannins, flavonoids, glycosides, saponins and volatile oils. Antioxidant activity was exhibited by the plant extract with the IC_{50} of 0.048 mg/ml and 0.051 mg/ml in DPPH and hydroxyl radical scavenging activity respectively. The reducing power of the extract showed concentration-dependent antioxidant reaction. The total phenolic compounds detected in the plant extract was 170.85 mg/g dry weight expressed as gallic acid equivalent (GAE) and flavonoids content was 83.3 mg quercetin equivalent (QE)/g dry weight. In in vivo study, the extract showed a significant increase ($p<0.05$) in Superoxide dismutase (SOD) and reduced Glutathione (GSH) level while there is a significant decrease ($P<0.05$) in Malondialdehyde (MDA) level. This study suggests that the hydro-alcoholic extract of *S. tuberosa* exhibited antioxidant activity. In vitro tests indicated a good antioxidant reaction and the presence of strong antioxidant compounds. In vivo experiments further revealed powerful antioxidant enzyme functions. Thus, the usefulness of the plant as a medicinal plant is supported.

Keywords: antioxidant, oxidative stress, phytochemical screening, *Stemona tuberosa*, streptozotocin

Introduction

Oxidative stress is an imbalance between the production of highly reactive and unstable molecules known as free radicals and the cell's ability to counteract their deleterious effects using antioxidants.[1,2] The most significant free radicals produced during metabolic reactions in the body are the reactive oxygen species (ROS).[3] The most common examples of ROS that cause oxidative damage to nucleic acids, carbohydrates, lipids, and proteins are mainly hydroxyl radical, superoxide anion, and hydrogen peroxide.[4] Over-production of these free radicals and inadequate availability of cellular antioxidants can cause oxidative damage which is the cause of various diseases like cancer,

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diabetes mellitus, atherosclerosis, arthritis, anemia, ischemia, asthma, inflammation, aging, immunosuppression and neurodegenerative disorders.[5]

The body has antioxidant defense systems that protect the cells against the toxic effects of ROS.[6] These antioxidants can naturally degrade and scavenge free radicals, thereby inhibiting oxidative damage caused by ROS.[7] These defense systems are mainly of two types - enzymatic (superoxide dismutase, glutathione peroxidase, catalase) and non-enzymatic (vitamin C and vitamin E, minerals, thiol reduced glutathione, polyphenols, etc.). Sometimes enzymatic antioxidants in the body are not sufficient or efficient enough to prevent the increasing ROS, necessitating the use of exogenous antioxidants from food supplements and medicinal plants.[8]

Stemona tuberosa Lour. is a wild asparagus belonging to the family of Stemonaceae. It is found in India, China, Australia, South East Asia, and Bangladesh at altitudes of 300—2300 m.[9] The main chemical constituents of *S. tuberosa* which have been identified are alkaloids, stilbenoids, and tocopherols. Traditionally, the roots of this plant have been used in the management of respiratory diseases, skin diseases, gynecological disorders, mental disorders, and jaundice.[10,11] Thus, it is important to understand the underlying pharmacological properties of the plants. Hence, the present study was designed to determine the *in vitro* and *in vivo* antioxidant activity of the hydro-alcoholic extracts of the roots of *S. Tuberosa*. The plant extract was also subjected to phytochemical screening to determine the types of secondary metabolites.

Materials and Methods

Chemicals and Reagents

Butylated hydroxy anisole (Merck Life Science Pvt. Ltd., India), 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, India), Ascorbic acid (Sigma-Aldrich, India), trichloroacetic acid (Merck Life Science Pvt. Ltd., India), thiobarbituric acid (Sigma-Aldrich,India), Folin-Ciocalteu reagent (Sigma-Aldrich,India), gallic acid (Sigma-Aldrich,India), quercetin (Sigma-Aldrich,India), streptozotocin (Sigma-Aldrich, India), lipid peroxidation assay kit (Sigma-Aldrich,India), glutathione assay kit (Sigma-Aldrich, India), and superoxide dismutase determination kit (Sigma-Aldrich,India). All the chemicals and reagents used were of analytical grade.

Collection and Identification of Plants

Stemona tuberosa was collected from Saitual, Mizoram, India on 19th April, 2023. A herbarium sheet of the plant was prepared and it was identified by the Botanical Survey of India, Shillong as *Stemona tuberosa*, family- Stemonaceae, with identification no. BSI/ERC/ Tech/2023-24/1084, dated 13/06/2023. It was later deposited in the Department of Pharmacy, RIPANS, Aizawl, Mizoram, India.

Extraction of the Plant Materials

Extraction of the roots of *S. tuberosa* was performed according to the methods described in Echegoyen et al.[12]. The roots of the plants were adequately cleansed with distilled water, shade-dried and ground into coarse powder. Maceration of the powdered dried roots (1000 g) was performed using hydro-alcoholic solution-ethanol/water (70:30, v/v) for 72 h in a flask. The extract was decanted and filtered using muslin cloth followed by Whatman No. 1 filter paper to obtain a clear extract. It was concentrated at 50 °C using a rotary evaporator and lyophilized to fine powder using a lyophilizer. The final extract was then stored in a refrigerator maintained at 4 °C for further use.

The percentage yield of the extract was calculated using the formula:

$$\text{Percentage yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \text{ x } 100$$

Preliminary Phytochemical Screening

The secondary metabolites present in *S. tuberosa* were determined using standard phytochemical screening procedures.[13] Various phytochemical testing were performed on the hydro-alcoholic extracts to detect the presence of the following phytoconstituents: alkaloids, carbohydrates, proteins and amino acids, steroids and triterpenoids, phenols and tannins, flavonoids, glycosides, volatile oil, fats and fixed oils.

In vitro Antioxidant Activities

DPPH radical scavenging activity

DPPH scavenging activity was performed by following the methods described by Blois[14] with minor modification using butylated hydroxyl anisole (BHA) as a reference standard. The root extract was dissolved in water to get a series of sample solutions with different pre-determined concentrations. DPPH was dissolved in methanol to make 0.1 mM solution and 0.5 ml of the solution was mixed with 3 ml of the plant extract of various concentrations and incubated at 37 °C for 30 minutes. The same procedure was also followed for standard solutions of similar concentrations. Absorbance was taken for each solution and

measured at 517 nm by using a UV–Vis spectrophotometer (Evolution 201, Thermo Scientific). Control reading was also taken.

The % inhibition was found out by using the formula:

$$\% \text{ inhibition} = (Abs_C - Abs_E / Abs_C) \times 100$$

where, Abs_C is absorbance of the control and Abs_E is absorbance of the plant extract.

IC_{50} was then estimated for the plant extract and standard drug from the graph by plotting the concentration on the X-axis and % inhibition on the Y-axis.

Hydroxyl Radical Scavenging Activity

Hydropxyl radical scavenging activity was performed as per the modified method[15] using the standard drug-ascorbic acid. 1 ml of various concentrations of extract and standard solutions were prepared separately. Then, 0.33 ml of phosphate buffer (pH 7.4), 0.1 ml of EDTA (1 mM), 0.01 ml of ferric chloride (10 mM) and 0.36 ml of deoxyribose (10 mM) were added to each of the solutions. 0.1 ml of ascorbic acid (2 mM) and 0.1 ml of hydrogen peroxide (10 mM) were then added to the mixtures to start the reaction. They were then incubated at 37 °C for 1 hour. 1 ml of 10% trichloro acetic acid and 1 ml of 0.5% thiobarbituric acid were then added to 1 ml of the incubated solution to develop the pink chromagen. The absorbance of the solutions was measured at 532 nm by using a UV-Vis spectrophotometer. The % inhibition was calculated by using the formula:

$$OH^- \text{ scavenged (\%)} = (Abs_C - Abs_E)/Abs_C \times 100$$

where, Abs_C is absorbance of the control and Abs_E is absorbance of the sample.

IC_{50} was then estimated for the plant extract and standard drug from the graph by plotting the concentration on the X-axis and % Inhibition on the Y-axis.

Determination of Reducing Power

The reducing power of *S. tuberosa* was determined by following the method mentioned by Malsawmtluangi et al.[16] using standard drug ascorbic acid. A volume of 2.5 ml phosphate buffer (6.6 pH) and 2.5 ml of 1% potassium ferricyanide solution were added to 1 ml of various concentrations of the plant extract. They were incubated for 30 minutes at 50 °C and the reaction was stop by adding 2.5 ml of 10% trichloroacetic acid. The solutions were then centrifuged for 10 minutes at 3000 rpm and 2.5 ml of the supernatant was taken out. 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride solution were added to the supernatant. The same procedure was also followed for the standard solutions of similar concentrations. Absorbance of the solutions was determined at 700 nm by using a UV-Vis spectrophotometer.

Determination of Total Phenolic contents

The total phenolic contents of the plant extract were estimated using the standard drug gallic acid.[17] A volume of 1 ml of the plant extract was mixed with 5 ml of Folin-Ciocalteau reagent. After 3 minutes, 4 ml of sodium carbonate solution was added, and the solution was allowed to stand at room temperature for 1 hour. Absorbance of the solutions was determined at 725 nm by using UV-Vis spectrophotometer, From the standard curve of gallic acid prepared in various concentrations, the total phenolic compound present in the plant extract was determined and then, expressed as gallic Acid equivalent (mg) of the dried extract.

Determination of Total Flavonoids Contents

The total flavonoids contents of the extract were estimated following Hapsari et al.[18] with minor modifications using quercetin as a standard drug. A volume of 2 ml of the root extract was mixed with 5% sodium nitrite (3 ml) and 10% aluminium chloride (0.3 ml). The solution was allowed to stand for 6 minutes, then, 1 M sodium hydroxide (2 ml) solution was added and the volume was adjusted up to 10 ml with distilled water. The solution was then kept for 30 minutes in a dark room. The absorbance reading of the solution was determined at 510 nm by using a UV-Vis spectrophotometer. From the standard curve of quercetin prepared in various concentrations, the total flavonoids content of the plant extract was determined and then, expressed as milligrams of quercetin equivalent (QE) of the dried extract.

Experimental Animals

In vivo antioxidant studies were performed on albino Wistar rats (120–150 g). The animals were housed in standard laboratory conditions (25 ± 2 °C), with a 12-hour light and dark cycle and supplied with a standard pellet diet and water *ad libitum*. All the experiments were performed as per the approval and guidelines of the Institutional Animal Ethics Committee (IAEC) of the Regional Institute of Paramedical and Nursing Science (RIPANS) with approval no. IAEC/RIPANS/36 for *in vivo* anti-oxidant activity.

Acute Toxicity Study

The acute toxicity study of the plant extract was performed on albino Wistar rats (female) according to Organization for Economic Co-operation and Development (OECD) guidelines no. 425. The animals were fasted overnight before the

administration of the extract but had access to water. A limit test was performed at 2000 mg/kg p.o. as a single dose and the animals were kept under observation for 14 days. During this period, observations were made for mortality, behavioral, neurological, and any other abnormalities.

In vivo Antioxidant Activity

In vivo antioxidant activity of the plant extract was evaluated following Sanilkumar and Muthu[19], using albino Wistar rats. Thirty-six male rats were randomly divided into six groups of six animals each, and were given treatment for 14 days. Group 1 served as a normal control group and received normal saline. Oxidative stress was induced in Group 2 to Group 6 animals with streptozotocin (STZ) at a dose of 60 mg/kg. Group 2, the STZ control group was also given normal saline. Group 3 and Group 4 were administered with glibenclamide (10 mg/kg) and ascorbic acid (50 mg/kg) respectively. Group 5 and Group 6 were treated orally with the hydroalcoholic extract of *S. Tuberosa* at doses of 200 and 400 mg/kg respectively. On the 15th day, the animals were sacrificed, and the liver and kidney of each animal were dissected out, washed in ice-cold saline, and homogenized in Tris HCL buffer (pH 7.4), to prepare 10% (w/v) homogenates. The homogenates were centrifuged at 4000 rpm at 4 °C for 15 minutes. The supernatants were collected and were further used for biochemical analysis. Malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) activities were measured in an ELISA microplate reader (BioTek - EPOCH/2) using Sigma – Aldrich Assay kit manual following the manufacturer's instructions.

Statistical Analysis

Statistical analysis of the data was done using GraphPad InStat software (version 3, ISS, Rome, Italy). All the values of the results were expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA followed by the Tukey-Kramer multiple comparison test. Values of $p<0.05$ were considered as significant.

Results

Extraction of Crude Drug

The total yields and percentage of the plant extracts were 190.35 gm and 19.035 % respectively.

Preliminary Phytochemical Screening

Table 1 shows the results of phytochemical screening of the plant extract. The results of the phytochemical testing indicated the presence of alkaloids, carbohydrates, proteins and amino acids, steroids, triterpenoids, phenols, tannins, flavonoids, glycosides and volatile oils.

In vitro Antioxidant Activity

DPPH radical scavenging activity

The scavenging activity of DPPH radical by the hydro-alcoholic extract of *S. tuberosa* was found to be in a concentration-dependent trend as shown in Figure 1. Butylated hydroxyl anisole exhibited higher DPPH radical scavenging activities than the hydro-alcoholic plant extract. The concentration of the plant extract to scavenge 50% of the DPPH radical is called IC_{50}. The IC_{50} of the extract of *S. tuberosa* exhibited 0.048 mg/ml as compared to standard drug, i.e. BHA which exhibited 0.033 mg/ml.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was found out by observing the competition between the plant extract and deoxyribose for hydroxyl radical, which then, attacked deoxyribose, resulting in the formation of thiobarbituric acid reacting substance. The scavenging effect of the plant extract was seen at all the tested concentrations, which increased in an increase in the extract concentration (Figure 2). The IC_{50} of the standard drug, i.e. ascorbic acid and plant extract were 0.037 and 0.051 mg/ml respectively.

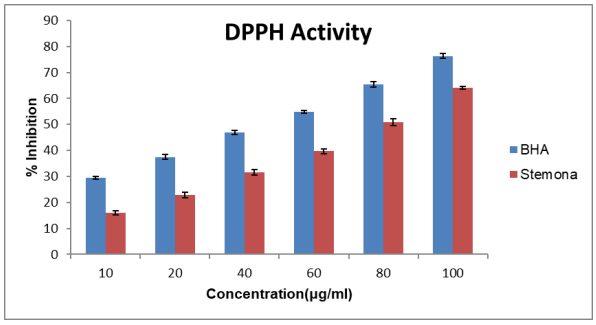

Figure 1. DPPH radical scavenging activity of *S. tuberosa* extract

Determination of Reducing Power

The reducing power of the *S. tuberosa* extract increased with an increase in its concentration indicating its ability to terminate radical chain reaction. The reducing power of the standard drug, i.e. ascorbic acid was found to be higher compared to the sample. However, the antioxidants present in the plant extract cause the reduction of Fe^{3+} to Fe^{2+} and thus prove its reducing power.

Table 1. Phytochemical screening of hydroalcoholic extract of *Stemona tuberosa*

Sl. No.	Test	Observation	Result
1	**Alkaloids** i) Hager's test	Yellow coloured precipitate was formed	+
	ii) Mayer's test	Cream coloured precipitate was formed	+
	iii) Dragendroff's Test	Orange-brown precipitate was formed	+
	iv) Wagner's Test	Reddish-brown precipitate was formed	+
2	**Carbohydrates** i) Molisch Test	Violet ring was formed	+
	ii) Benedict's test	Red coloured precipitate was formed	+
	iii) Fehling's Test	Brick red precipitate was formed	+
3	**Proteins and Amino acids** i) Xanthoproteic Test	Yellow colour was formed	+
	ii) Ninhydrin Test	Violet colour was formed	+
	iii) Biuret Test	Violet colour was formed	+
4	**Steroids and Terpenoids** i) Salkowski test	Red colour was formed	+
	ii) Libermann-Burchard Test	Brown ring at the junction of two layers and green color at the upper layer was formed	+
5	**Phenols and Tannins** i) Ferric chloride test	Blue colour was formed	+
	ii) Gelatin test	White precipitate was formed	+
	iii) Chlorogenic acid test	Green colour was formed	+
6	**Flavonoids** i) Shinoda Test	Pinkish red colour was formed	+
	ii) Alkaline Reagent Test	Intense yellow colour formed turned to colour-less on the addition of a few drops of dilute acid	+
	iii) Lead acetate Test	Yellow colour precipitate was formed	+
	iv) Sulphuric Test	Orange colour was formed	+
7	**Glycosides** i) Borntrager's Test	Pinkish red colour was formed	+
	ii) Liebermann's Test	Colour change from violet to greenish blue	+
	iii) Keller-killiani test	Brown ring was formed	+
8	**Volatile Oil**	Red colour was obtained by globules	+
9	**Fats and Fixed Oil**	No reaction	-

(+) = Present, (-) =Absent

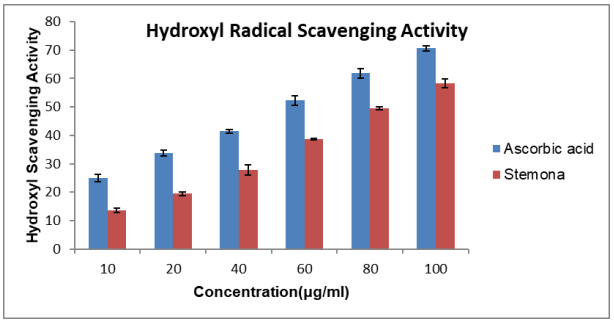

Figure 2. Hydroxyl radical scavenging activity of *S. tuberosa* extract

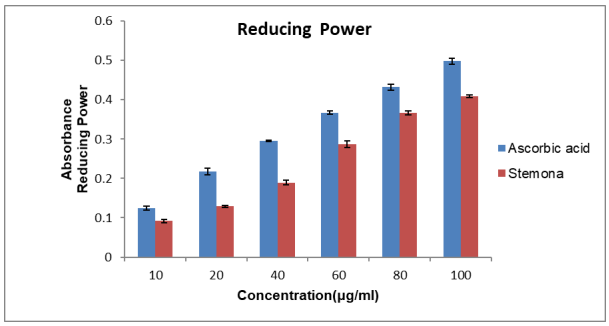

Figure 3: Reducing Power Activity of *S. tuberosa* extract

Total Phenolic and Total Flavonoid Contents

The total phenolic compounds detected in the extract of *S. tuberosa* was 170.85 mg/g dry weight expressed as gallic acid equivalent (GAE) and flavonoid content was found to be 83.3 mg quercetin equivalent (QE)/g dry weight.

Acute Oral Toxicity Study

An acute toxicity testing was done by following the limit test of OECD 425 guidelines. Observations on the animals were made for 14 days. 2000 mg/kg of the plant extract did not produce any mortality to the animals. No significant changes were observed in the behaviour, body weight and other physical examination of the animals. The observations indicate that the plant extract is well tolerated and non-toxic to the test model.

In vivo Antioxidant Activities

Estimation of Malondialdehyde (MDA)

The concentration of MDA in various groups of animals is shown in Table 2. The concentration of MDA in Group 2 (STZ Control Group) was increased as compared with the Normal Group, which showed that oxidative stress was induced by streptozotocin. The concentration of MDA in the liver of Group 4 treated with ascorbic acid was restored to the normal value, which showed that ascorbic acid has higher antioxidant activity than glibenclamide in oxidative stress conditions. The results also revealed that the plant extract reduced MDA concentration in experimental group animals, showing that the plant possessed antioxidant activity.

Table 2. Effects of MDA in liver and kidney in Control and Experimental Groups.

MALONDIALDEHYDE (MDA) (nM/µl)			
GROUP		LIVER	KIDNEY
1.	Normal Control	0.074±0.0238	0.047±0.009
2.	STZ Control	$0.17\pm0.029^{++}$	$0.169\pm0.029^{+++}$
3.	STZ + Glibenclamide	$0.085\pm0.015^{*}$	$0.072\pm0.016^{**}$
4.	STZ + Ascorbic acid	$0.075\pm0.015^{**}$	$0.054\pm0.012^{***}$
5.	STZ + Extract LD	$0.089\pm0.011^{*}$	$0.075\pm0.007^{**}$
6.	STZ+ Extract HD	$0.081\pm0.013^{**}$	$0.068\pm0.001^{**}$

All values are mean ± SEM, n=6. ***P<0.001 vs. STZ control, **P<0.01 vs. STZ control, *P<0.05 vs. STZ control, $+^{++}P$<0.001 vs. normal control, ^{++}P<0.01 vs. normal control. STZ= Streptozotocin, LD=Low dose, HD= High dose

Table 3. Effects of SOD in liver and kidney of Control & Experimental Groups of rats.

SOD Activity (% Inhibition)			
Group		Liver	Kidney
1.	Normal Control	50.66±1.83	39.5±1.82
2.	STZ Control	$31\pm1.39^{+++}$	$26.33\pm1.52^{+++}$
3.	STZ + Glibenclamide	$42.16\pm1.92^{**}$	$36.5\pm1.94^{**}$
4.	STZ + Ascorbic acid	$55.83\pm1.92^{***}$	$42.66\pm2.36^{***}$
4.	STZ + Extract LD	38.16 ± 2.05^{ns}	33.83 ± 1.32^{ns}
5.	STZ+ Extract HD	$46\pm1.84^{***}$	$37.83\pm1.85^{**}$

All values are mean ± SEM, n=6. ***P<0.001 vs. STZ control, **P<0.01 vs. STZ control, ^{+++}P<0.001 vs. normal control, ns= not significant, STZ= Streptozotocin, LD=Low dose, HD= High dose.

Estimation of Superoxide Dismutase (SOD)

The concentration of superoxide dismutase (SOD) in various groups of animals is shown in Table 3. SOD activity was the lowest in Group 2 animals. High doses of the plant extract and standard drugs which were administered in the experimental group significantly increases the SOD activity.

Table 4. Reduced GSH concentration in the liver and kidney of rats.

Reduced GSH Conc. (nm/ml)			
GROUP		**Liver**	**Kidney**
1.	Normal Control	2822±159.71	1825.33±53.45
2.	STZ Control	1344.33±63.93^{+++}	1148.50±79.51^{+++}
3.	STZ + Glibenclamide	1761.33±89.21^{ns}	1483.33±67.74^{ns}
4.	STZ + Ascorbic acid	4144.83±77.79***	3952.50±30.83***
5.	STZ + Extract LD	2926.16 ±78.23***	1993.66 ±73.15***
6.	STZ+ Extract HD	3938.50±140.03***	2123.83±43.95***

All values are mean ± SEM, n=6. ***P<0.001 vs. STZ control, ^{+++}P<0.001 vs. normal control, ns= not significant, STZ= Streptozotocin, LD=Low dose, HD= High dose

Estimation of Reduced Glutathione (GSH)

Table 4 shows the concentration of GSH in the liver and kidney of control and experimental groups of rats. The decrease in GSH level was observed in group 2, i.e. STZ control group. Administration of the plant extract and ascorbic acid brought the reduced GSH levels higher in experimental animals as compared with the control animals.

Discussion

Plants have been utilized to mitigate or treat diseases in various traditional medicine systems.[20] Pharmacological studies have shown the value of medicinal plants as a potential source of bioactive compounds.[21] The beneficial medicinal effects of plant extracts resulted from the combination of the secondary metabolites. Phytochemicals are known to possess various pharmacological activities like anticancer, antioxidant, antibacterial, antifungal, hepatoprotective, antidepressant, antidiabetic, anti-inflammatory, etc..[22] This study revealed that the hydro-alcoholic extracts of the plant contained several phytochemicals including phenols, flavonoids, and saponins which are related with the antioxidant activity of the plant. The phytochemicals might exhibit their antioxidant activity via stabilizing or scavenging free radicals through hydrogenation or complexion with oxidizing species.[23]

DPPH and hydroxyl radicals are free radicals and their scavenging activities are commonly employed to estimate the antioxidant activity of many plant extracts. With the increase in the concentration of plant extract, the percentage of free radical inhibition increases. In this study, the IC_{50} of the standard drug is lower than the plant extract, showing the standard drug has higher potency than the extract. The reducing power activity is mainly used to find out the capacity of an antioxidant to donate an electron. In this activity, the antioxidants in the extract resulted in a reduction of the ferric cyanide complex to the ferrous cyanide form. Higher is the absorbance of the solution; higher would be the reducing power. The reducing power of *S. tuberosa* increased as the concentration increases, which indicated that the radical chain reaction was stopped by some compounds present in the extract. Phenolic compounds like flavonoids are known to possess a wide range of antioxidant activity. The primary mechanism underlying the antioxidant activity of phenolic compounds is their redox property, which can help absorb and neutralize free radicals, quenching singlet and triplet oxygen, or dissolving peroxides.[24]

Free radicals give rise to lipid peroxidation which is the degradation of lipids as a result of oxidative damage. Malondialdehyde is the product of such peroxidation and its level is used as a biomarker of oxidative stress. An increase in free radicals causes the overproduction of malondialdehyde.[25] Superoxide dismutases constitute a very important antioxidant defense against oxidative stress in the body. This antioxidant enzyme forms the first line of defense against reactive oxygen species-mediated injury and decreases the free radical.[26] The non-enzymic antioxidant, glutathione plays a crucial role in various biological processes and protects the cells against oxidation. Glutathione is mainly concerned with the removal of free radical species like hydrogen peroxide, superoxide radicals and alkoxy radicals, and also acts as a substrate for glutathione peroxidase and glutathione-s-transferase. This study indicated that the plant extract markedly lowered lipid peroxidation, increased reduced glutathione concentration and superoxide dismutase activities in the STZ-induced oxidative stress animals.

Conclusion

The results of the *in vitro* and *in vivo* antioxidant studies indicated that *S. tuberosa* extract have effective antioxidant activity, which may be due to the secondary metabolites of the

plants. Thus, the present data revealed that the plant extract can be used as a good source of natural antioxidants for the treatment and prevention of various diseases. The current study also gives evidence that streptozotocin has cytotoxic effects against the pancreas and can induced hyperglycemia due to the generation of ROS. Reduced activities of endogenous antioxidants with elevation of lipid peroxidation marker (MDA) have been observed in STZ control rats. So, these findings suggested that *S. tuberosa* can act as an antioxidant agent in *Diabetes mellitus*.

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Conflict of Interest

The author declares that there is no conflict of interest.

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