Abstract
This study evaluates the phytochemical constituents, free radical scavenging activities and antioxidative potential of the tuber extracts of *Stemona tuberosa* (ST). The preliminary phytochemical screening revealed the presence of alkaloids, cardiac glycosides, saponins, steroids, tannins and terpenoids from various solvent extracts of ST. The methanolic extract of ST showed the highest phenolic (715.20 ± 2.42 mg GAE/g dry extract) and flavonoid (3864.25 ± 7.54 mg quercetin/g dry extract) contents. *S. tuberosa* extracts were analyzed for their scavenging activities based on 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2’-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and superoxide anions (O₂⁻) in a cell free system. Different extracts of ST inhibited the generation of DPPH, ABTS and O₂⁻ in a concentration dependent manner. Among the various extracts of ST, the methanolic extract showed the highest scavenging activities for ABTS and O₂⁻ with IC₅₀ of 36.20 ± 0.832 µg/ml and 98.93 ± 3.37 µg/ml respectively. The scavenging activity of methanolic extract for ABTS and O₂⁻ was significantly higher than the standard ascorbic acid. However, chloroform extract was found to possess the highest scavenging activity for DPPH with IC₅₀ of 7.36 ± 0.081 µg/ml. The total reducing power of ST extracts was also determined by measuring the transformation of Fe³⁺ into Fe²⁺ and the methanolic extract was found to exhibit the highest reducing power. The extracts were also analyzed for their anti-haemolytic activity and inhibitory effect on lipid peroxidation in an *ex vivo* condition using mice erythrocyte and liver, respectively. The anti-haemolytic activity of ST extracts also increased with the increase in concentration of the extract. Chloroform extract was found to possess the highest anti-haemolytic activity with 68.81 % inhibition followed by methanolic extract (38.57 %) and aqueous extract (20.81 %). Methanolic extract showed the highest inhibitory activity on lipid peroxidation (80.5 %) followed by chloroform extract (67.8 %) and then aqueous extract (62.63 %). Our study suggests that ST extracts have free radical scavenging and antioxidative potential, probably due to their high phenolic and flavonoid contents.

**Keywords:** *Stemona tuberosa*, Free radicals, Antioxidants, Lipid peroxidation.

Introduction
During normal cellular metabolism, molecular oxygen results in production of reactive oxygen species (ROS) such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH) and singlet oxygen (¹O₂). At low to moderate concentrations, ROS are fundamental in modulating various physiological functions of the body, representing an essential part of aerobic
life and metabolism (1 and 2). However, excessive generation of ROS hampers the antioxidant defense systems of the body leading to a condition called ‘oxidative stress’ ensuing tissue damage, and have been reported to be associated with diseases including coronary heart disease, neurodegenerative disorders, diabetes, arthritis, inflammation, lung damage and cancer (3 and 4). Certain plants have been reported to contain various phytochemicals that are generally non-toxic, act as natural antioxidants and useful for the treatment of different diseases (5). Since, antioxidants are capable of preventing oxidative damage, use of plants and plant-derived products as a source of natural antioxidants has been employed as a conventional method for maintaining oxidative balance in the body.

_Stemona tuberosa_ is an elegant plant belonging to the family Stemonaceae. It is native to China, Southeast Asia, North-east India and New Guinea, and is one of the 50 fundamental herbs used in traditional Chinese medicine (6). Roots of _S. tuberosa_ has been traditionally used for the treatment of cough and chest pains (7), infestations with lice and treatment of enterobiasis, and in revitalization of the body and provision of sexual stimulant (8). Among the genus _Stemona_, _S. collinsae_ has been reported for its antifungal property, _S. japonica_ for its antimicrobial activity (9). Other species such as _S. sessifolia_ and _S. curtisii_ have also been reported to contain certain alkaloids that may possess antifungal, anti-inflammatory and antitussive activities (10). Antioxidative potential is widely used as a parameter to assess medicinal property of natural products or the bioactive components of plants. _In vitro_ screening methods have been commonly used for chemical elucidation and pharmacological investigations of medicinal plants (11). Therefore, the present study aimed to evaluate the free radical scavenging activity and antioxidant potential of the tuber extracts of _Stemona tuberosa_.

**Materials and Methods**

**Chemicals used**: Gallic acid, quercetin dihydrate, methanol, ferric chloride, sodium nitrite, nitrobluetetrazolium (NB), nicotinamide adenine dinucleotide (NADH), phenazinemethosulfate (PMS), 2-deoxyribose, 2,2’-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), di-sodium hydrogen phosphate, potassium persulfate and hydrogen peroxide (H$_2$O$_2$) were obtained from HiMedia Laboratories Pvt., Ltd. (Mumbai, India). 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) and thiobarbituric acid (TBA), were obtained from Sigma Aldrich Inc (Louis, Germany). Trichloroacetic acid (TCA), Folin-ciocalteau’s reagent, sodium hydroxide, sodium carbonate, ascorbic acid and ferrous sulphate were obtained from SD fine—chem Ltd. (Mumbai, India). Alluminium chloride and sodium di-hydrogen phosphate were obtained from Merck Specialties Pvt., Ltd. (Mumbai, India). Ethylene diame tetraacetic acid (EDTA) was obtained from Qualigens Fine Chemicals (Mumbai, India). Potassium ferricyanide was obtained from LobaChemie Pvt., Ltd. (Mumbai, India).

**Preparation of extracts**: The plant _Stemona tuberosa_ (ST) was identified and collected from Phura, Siaha District, Mizoram. The roots were washed and chopped into smaller pieces and then shade dried at room temperature. The dried roots were grounded to powder using a mixer grinder and then sequentially extracted with petroleum ether, chloroform, methanol and distilled water based on their increasing polarity using Soxhlet apparatus at their respective boiling points for a minimum of 40 cycles each. The liquid extracts were filtered, allowed to evaporate and finally freeze dried so as to obtain a fine powder of the extract. Henceforth, the chloroform extract, methanolic extract and aqueous extract of ST will be called as STCE, STME and STAE respectively.

**Phytochemical analysis**: The following phytochemicals were screened for their presence in various extracts of _S. tuberosa_ using standard protocols.

**Alkaloids**: The presence of alkaloids in ST extracts was confirmed by employing the Dragendorff’s test. Briefly, 0.1 g of different extracts of ST was mixed with 0.5 ml of
Dragendorff’s reagent. The development of reddish brown precipitate indicates the presence of alkaloids (12).

**Cardiac glycosides**: ST extract (0.1 g) was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution with an underlaying of 1 ml of concentrated H₂SO₄. The appearance of brown ring at the interface indicated the presence of deoxysugar, which is a characteristic of cardenolides (13).

**Saponins**: ST extracts (0.1 g) was mixed with 3 drops of olive oil and shaken vigorously for few min. The formation of a fairly stable emulsion indicated the presence of saponins (13).

**Steroids**: The presence of steroid in various extracts of ST was determined by Salkowski’s test. Briefly 0.1 g of ST extract dissolved in their respective solvents was mixed with a few drops of concentrated H₂SO₄. The development of red colour at lower layer indicated the presence of steroids (12).

**Tannins**: Presence of tannin was determined by Ferric chloride test. Briefly, 0.1 g of ST extract was dissolved in their respective solvents and a few drops of 0.1% ferric chloride were added. The formation of brownish green or a blue-black colour indicated the presence of tannins (13).

**Terpenoids**: The presence of terpenoids in various extracts of ST was detected using Salkowski’s test. 5 ml of each extract (0.1 g/ml) was mixed with 2 ml of chloroform followed by careful addition of 3 ml concentrated H₂SO₄ so as to allow the formation of a layer. The formation of a reddish brown colour at the interface confirmed the presence of terpenoids (12).

**Phlobatannins**: Different extracts of ST (0.1 g) was boiled in 1% aqueous hydrochloric acid and deposition of a red precipitate indicated the presence of phlobatannins (13).

**Quantitative estimation of phytochemicals**

**Determination of total phenolic content**: The total phenolic content of various extracts of ST was determined using the method described earlier (14). Briefly, 5 ml of Folin-ciocalteau reagent (diluted ten-fold) was mixed with 1 ml of plant extracts (STCE, STME, STAE) dissolved in their respective solvent, at the concentration ranging from 0.25-8.0 mg/ml. Sodium carbonate (4 ml, 0.115 mg/ml) was added to the mixture after 5 min of incubation at room temperature. Then, the mixture was incubated for 2 hr in the dark at room temperature followed by measuring the absorbance at 765 nm using UV-Visible spectrophotometer (SW 3.5.1.0. Bio-spectrometer, Eppendorf, India Ltd., Chennai) Calibration curve was also prepared by mixing methanolic solution of gallic acid (1 ml, 0.25–4.0 mg/ml) with the reagents above and absorbance was measured at 765 nm. All determinations were carried out in triplicate. The total phenolic content in each extract was expressed as gallic acid equivalents (GAE) mg/g of the dry extract.

**Determination of the total flavonoids**: Total flavonoid content was determined using the method described earlier (15). Briefly, 0.25 ml of different fractions of the extract (0.25–4.0 mg/ml; dissolved in respective solvent) and quercetin standard solution was mixed with 1.25 ml of distilled water followed by the addition of 75 μl of 5% (w/v) sodium nitrite solution. After few min, 150 μl of 10% (w/v) aluminum chloride solution was added and allowed to stand for further 5 min before the addition of 0.5 ml of 1 M NaOH. The mixture was then made up to 2.5 ml with distilled water and mixed well. Absorbance was measured immediately at 510 nm. The result was expressed as quercetin equivalents (mg/g extract) All estimations were performed in triplicate.

**In vitro antioxidant assays**

**DPPH radical scavenging activity**: DPPH radical scavenging activity was carried out according to the method described earlier (16) with slight modifications. To different concentrations of various extracts of ST (0.5 ml, 1-400 μg/ml), 1 ml of methanolic solution of 0.1 mM DPPH was added. The mixture was then allowed to stand in the dark for 30 min and absorbance was measured at 523 nm. Methanol

Free radical scavenging activities and antioxidative potential of *Stemona tuberosa*. 
was utilized as the baseline correction. The results were compared with that of the control prepared as above without sample. The antioxidant activity of the extract was expressed as IC\textsubscript{50}, the concentration (µg/ml) of extract that inhibited 50% of DPPH radicals. Ascorbic acid was used as the positive control and each study was performed in triplicate. The scavenging activity was then estimated using the formula:

\% scavenging = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100

Where, \( A_{\text{blank}} \) is the absorbance of the control reaction (containing all reagents except the test compound) and \( A_{\text{sample}} \) is the absorbance of the test compound.

**Superoxide radical scavenging activity**:
Superoxide scavenging activity was determined by the nitroblue tetrazolium (NBT) reduction method (17) with minor modifications. To the reaction mixture containing 0.2 ml of NBT (1mg/ml in DMSO) and 0.6 ml of extract (1-800 mg/ml), 2 ml of alkaline DMSO (1 ml DMSO in 5 mM NaOH) was added to give a final volume of 2.8 ml. The absorbance was recorded at 560 nm. The blank consisted of pure DMSO instead of alkaline DMSO. Ascorbic acid was used as the standard and the ability of ST extracts to scavenge the superoxide radical was calculated using the formula:

\% scavenging = \left( \frac{A_e - A_o}{A_e} \right) \times 100

Where, \( A_o \) is absorbance without sample and \( A_e \) is absorbance with sample.

**ABTS radical scavenging activity**:
The scavenging activity of ST extracts for ABTS radical was determined using the method of Re et al. (18). A stock solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution. The solution was incubated at room temperature in the dark for 12 hr to yield a dark-coloured solution containing ABTS\textsuperscript{+} radicals. A working solution was prepared freshly before each assay by diluting the stock solution with 50% methanol for an initial absorbance of about 0.700 (±0.02) at 745 nm. The scavenging activity was then assessed by mixing 150 µl of different fractions of various extracts (1-200 µg/ml, dissolved in their respective solvents) with 1.5 ml of ABTS working standard. The decrease in absorbance was measured immediately at 745 nm. Each experiment was done in triplicate. Ascorbic acid was used as positive control. The scavenging activity was then estimated based on the formula:

\% scavenging = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100

Where, \( A_{\text{blank}} \) is the absorbance of the control reaction (containing all reagents except the test compound) and \( A_{\text{sample}} \) is the absorbance of the test compound.

**Reducing power**:
The reducing power of ST extracts was determined using the method described earlier (19) with slight modifications. Different extracts of ST dissolved in their respective solvent (1-1000 mg/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 20 min after which 2.5 ml of 10% TCA was added. The mixture was then centrifuged at 3000 rpm for 10 min and 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 1% ferric chloride solution. Absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increase in reducing power of the extract.

**Ex vivo antioxidant assay**

**Anti-hemolytic activity**:
The antioxidant activity of different extracts of ST was measured according to the inhibition of erythrocyte hemolysis (20). Blood was collected from mice of same age group (10-12 w) and body weights (25-27 g) by means of heart puncture in heparinized tube. The mice erythrocyte hemolysis was performed with H\textsubscript{2}O\textsubscript{2} as free radical initiator. To 0.5 ml of 5% (v/v) suspension of RBC in PBS, 0.4 ml (0.5 mg/ml) of different extracts and 100 µl of 1 mol/L H\textsubscript{2}O\textsubscript{2} was added. The reaction mixture was shaken gently while being incubated at 37°C for 3 hr. After incubation the reaction mixture was again diluted with 4 ml of PBS and centrifuged at 2000
rpm for 10 min. The supernatant was collected and absorbance was measured at 540 nm. The rate of inhibition of erythrocyte hemolysis was calculated using the formula:

\[
\text{Inhibition rate} \% = \left[ 1 - \frac{(A1 - A2)}{A0} \right] \times 100
\]

Where, \(A0\) is the absorbance of control (without extract), \(A1\) is the absorbance in the presence of the extract and \(A2\) is the absorbance without sample (RBC).

**Lipid peroxidation inhibition assay:** Lipid peroxidation in mice liver was induced by \(\text{FeCl}_2 - \text{H}_2\text{O}_2\) (21). Briefly, liver was excised from mice and 1% liver homogenate was prepared. The liver homogenate was centrifuged at 3000 rpm at 4°C for 10 min and the supernatant was used for the assay. The supernatant (0.5 ml) was mixed with 0.5 ml (0.5 mg/ml) of extracts and then incubated at 37°C for 1 hr after mixing with 0.25 ml each of 0.5 mol/L \(\text{FeCl}_2\) and \(\text{H}_2\text{O}_2\). After incubation, the formation of malonaldehyde (MDA) was measured at 535 nm. The inhibitory effect was calculated as:

\[
\text{Inhibition rate} \% = \left[ 1 - \frac{(A1 - A2)}{A0} \right] \times 100
\]

Where, \(A0\) is the absorbance of control (without extract), \(A1\) is the absorbance in the presence of the extract whereas \(A2\) is the absorbance without liver homogenate.

**Animal model:** The inbred Swiss albino mice colony is being maintained under controlled conditions of temperature (22°C ± 5°C) and 12 h light-dark cycles (Frontier Euro Digital Timer, Taiwan) at the Animal Care Facility of the Department of Zoology, Mizoram University, Aizawl, India. The animals were fed with commercially available food pellets and water *ad libitum*. The animal care and handling was carried out according to the guidelines issued by World Health Organization, Geneva, Switzerland. The study was approved by the Institutional Animal Ethical Committee, Mizoram University, India vide approval No. MZU-IAEC/2018/09.

**Statistical analysis:** Data are expressed as mean ± standard error of mean. The IC\(_{50}\) values were calculated using Graph pad prism software ver. 6.0 by plotting the values against the log doses. One-way analysis of variance (ANOVA) was performed to test the significant variations on phytochemical contents and the antioxidant assays of various extracts followed by Tukey multiple comparison of means. SPSS ver.16.0 software (SPSS Inc, Chicago, Illinois, USA) and Graph pad prism software ver. 6.0 were used for the statistical and graphical evaluations. A p-value of less than 0.05 was considered statistically significant.

**Results and Discussion**

**Phytochemical analysis:** Qualitative screening revealed the presence of various active and naturally occurring phytochemicals such as alkaloids, cardiac glycosides, saponins, steroids, tannins and terpenoids in different extracts of *Stemona tuberosa* (Table 1). These phytochemicals belongs to polyphenolic compounds and have been reported to possesses numerous pharmacological values including anti-malarial (22), astringents (23), anti-inflammatory (24), anti-ulcer and antimicrobial activities (25).

**Total phenolic and flavonoid contents:** The total phenolic content of ST extracts increased in a concentration dependent manner (Fig. 1). At 8 mg/ml, STME has significantly higher (p<0.001) total phenolic content (715.20 ± 2.42 mg gallic acid equivalent/g of dry extract) than that of STCE (549.49 ± 16.67 mg gallic acid equivalent/g of dry extract) and STAE (367.92 ± 2.47 mg gallic equivalent/g of dry extract). The total flavonoid content of various extracts of ST also increased with the increase in concentration of the extracts (Fig. 2). At 8 mg/ml of extracts, STME has the highest (p<0.001) total flavonoid content (4890.13 ± 8.97 mg quercetin equivalent/g of dry extract) as compared to other fractions of the ST extracts (456.94 ± 13.70 mg quercetin equivalent/g of dry extract for STAE; 2902.48 ± 6.26 mg quercetin equivalent/g of dry extract for STCE). The present study indicated the presence of significant amounts of flavonoid and phenolic compounds in ST extracts. Phenolic compounds have been reported to show antioxidant activity by scavenging or stabilizing free radicals due to their conjugated
Table 1: Phytochemical screening of various extracts of S. tuberosa. (+’ indicates presence of phytochemicals and ‘–’ indicates absence of phytochemicals).

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Reagent</th>
<th>Colour Indication</th>
<th>STCE</th>
<th>STME</th>
<th>STAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s Reagent</td>
<td>Reddish brown precipitate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Glacial Acetic Acid Ferric Chloride Sulphuric Acid</td>
<td>Brown ring</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Olive oil</td>
<td>Whitish Emulsion</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Sulphuric Acid</td>
<td>Red Colour</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric Chloride</td>
<td>Brownish Green or blue-black</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Sulphuric acid</td>
<td>Reddish Brown</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>Hydrochloric acid</td>
<td>Red precipitate</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1: Phenolic content of various extracts of S. tuberosa determined as gallic acid equivalent. Values are expressed as Mean ± SEM, n=3. Different letters indicate significant variation.

Fig. 2: Flavonoid content of various extracts of S. tuberosa determined as Quercetin equivalent. Values are expressed as Mean ± SEM, n=3. Different letters indicate significant variation.
ring structures and presence of hydroxyl groups (26 and 27). Phenolic compounds have also been reported to exhibited antiallergenic, antimicrobial, anti-artherogenic, antithrombotic, anti-inflammatory, vasodilatory and cardioprotective effects (28 and 29). Similarly, flavonoids are also reported to have antioxidative action through scavenging or chelating process (30).

**In vitro antioxidant assay**

**DPPH radical scavenging activity**: Various extracts of ST showed a concentration dependent increase in the scavenging of DPPH radicals as indicated by the discoloration of DPPH. Maximum scavenging was observed at a concentration of 200 μg/ml for all the extracts. Log-doses of various extracts of *S. tuberosa* and standard ascorbic acid (ASA) were plotted against DPPH inhibition (%) for the calculation of IC$_{50}$ (Fig. 3). STCE showed the highest scavenging activity (lowest IC$_{50}$: 7.36 ± 0.08 μg/ml) followed by STME (IC$_{50}$: 13.46 ± 0.04 μg/ml) and STAE (IC$_{50}$: 18.41 ± 0.16 μg/ml). The IC$_{50}$ of STCE does not significantly differ (p > 0.05) from that of the standard ASA (IC$_{50}$: 6.57 ± 1.01 μg/ml) (Fig. 4). It has been reported that the reducing capability of the methanolic DPPH solution to a non-radical DPPH-H form by various compounds such as cystein, glutathione, ascorbic acid and tocopherol was due to their hydrogen donating ability (31 and 32). In the present study, various solvent extracts of ST effectively reduced the stable radical DPPH to the yellow-colored diphenyl-picrylhydrazine probably due to presence of certain active compounds that are capable of donating hydrogen to a free radical in order to remove electron.

**Superoxide radical scavenging activity**: Superoxide radical scavenging activity of various extracts of ST showed a concentration dependent inhibition of superoxide radical generation. Maximum O$_2^-$ scavenging activity was observed at a concentration of 800 μg/ml for STME and STAE. STME possessed the highest scavenging activity (IC$_{50}$: 98.93 ± 3.37 μg/ml) followed by STAE (241.83 ± 3.15 μg/ml) while the IC$_{50}$ of STCE was indeterminable within the given concentration (800 μg/ml). The IC$_{50}$ of STME and STAE are significantly higher (p < 0.01) than that of the standard ascorbic acid (IC$_{50}$: 262.20 ± 4.25 μg/ml) (Figure 6). Superoxide (O$_2^-$) radical serves as a precursor of most of the reactive oxygen species (33) and can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, which are very harmful to the cellular components and can initiate lipid peroxidation (34). Thus, neutralization of superoxide radical will inhibit the chain of ROS generation and protect the cells from oxidative stress. It has also been reported that antioxidant properties of some flavonoids are effective mainly through scavenging of superoxide anion radical (35). Thus, the presence of significant amount of flavonoids in ST extracts might be responsible for...
their scavenging activity against superoxide radical. Interestingly, the scavenging potentials of STME and STAE were even better than the standard ascorbic acid.

**ABTS radical scavenging activity**: ABTS•+ radical scavenging activity of ST extracts increased in a concentration dependent manner as indicated by discoloration of the ABTS•+, which was measured spectrophotometrically at 745 nm. Maximum scavenging activity was observed at a concentration of 200 μg/ml for all the extracts. Log-doses of various extracts of *S. tuberosa* and the standard ascorbic acid (ASA) were plotted against ABTS•+ inhibition (%) for the calculation of IC_{50} (Fig. 7). Among the various extracts, STME possessed the highest ABTS•+ scavenging activity (IC_{50}; 36.20 ± 0.83 μg/ml) followed by STAE (IC_{50}; 45.12 ± 0.79 μg/ml) and STCE (IC_{50}; 66.8 ± 1.04 μg/ml) (Fig. 8). The effectiveness of any compound in stabilization of the preformed ABTS•+ to ABTS depends on the molecular weight of phenolic compounds, the number of aromatic rings and nature of hydroxyl group’s substitution than the specific functional groups (36). Hence, the ABTS•+ scavenging activity of ST extracts might be due to the presence of high molecular weight phenolics such as catechin, and rutin derivatives.

**Reducing power**: The reducing power of various extracts of ST was determined by measuring the
transformation of Fe^{3+} to Fe^{2+}. The reducing activity of ST extracts increased in a concentration dependent manner (Figure 9). At 1000 ìg/ml, the highest reducing activity was shown by STME (1.975 ± 0.002) followed by STCE (1.952 ± 0.005) and STAE (1.016 ± 0.007). The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (37). The dose dependent increase in the reducing power of the ST extracts also suggested their potent antioxidant activity.

**Ex-vivo antioxidant assay**

**Anti-hemolytic activity** : Erythrocytes were considered to be the major target of free radicals, leading to membrane damage and consequently to hemolysis (38 and 39). The anti-hemolytic activity of various extracts of ST increased in a concentration dependent manner (Fig. 10). At 1 mg/ml, STCE possessed the highest (p < 0.001) inhibitory activity against erythrocyte hemolysis with an inhibition rate of 68.82 % followed by STME and STAE with the inhibition rate of 38.58 % and 20.81 % respectively (Fig. 10). The protective effect of ST extracts on erythrocyte hemolysis could be due to the presence of significant amounts of phenolic and flavonoid compounds. Certain phenolic compounds have been reported to partition in the cell membrane, hindering diffusion of free radicals and consequently decreasing the kinetics of free radicals reactions (40). In addition, flavonoids upon binding to the erythrocytes membrane inhibit lipid peroxidation and improved their integrity against cell lyses (41).
The study indicated that the tuber extracts of ST contains molecules that might interacts with the membrane lipids of erythrocyte membrane and providing protective action against hemolysis.

**Lipid peroxidation inhibition**: In biological systems, lipid peroxidation generates certain degradation products such as MDA that are considered to be an important cause of cell membrane destruction and cell damage (21). *S. tuberosa* extracts exhibited moderate activity against lipid peroxidation inhibition in liver homogenate. At a concentration of 0.5 mg/ml, inhibition rates were significantly different among various extracts of ST ($F_{2,6}=514.95$) and STME possessed the highest (p<0.001) inhibitory activity against lipid peroxidation with an inhibition rate of 80.55 % followed by STCE and STAE with the inhibition rate of 67.81 % and 62.64 % respectively (Fig. 11).

**Conclusions**

The present study revealed the presence of significant amounts of phenolic and flavonoid compounds along with various active polyphenolic compounds such as alkaloid, cardiac glycosides, saponins, steroids, tannins and terpenoids. This study also demonstrates that various solvent extracts of *Stemona tuberosa* exhibit a concentration dependent inhibition of free radicals, ferric reducing power, anti-hemolytic and inhibitory action against lipid peroxidation. The radicals scavenging activity and the antioxidative property of ST might be due to presence of significant phytochemical contents. However, an effort to understand the mechanism(s) through which ST exerts antioxidant activities need to be investigated further.

**References**


Antioxidant Capacities of Some Food Plants Wildly Grown in Ayvalik of Turkey. Food Science and Technology Research, 15: 59-64.


