

ORIGINAL CONTRIBUTION

Open Access

# Hepatoprotective and antioxidant activity of *Ipomoea staphylina* Linn



Ramachandran Jeyadevi<sup>1</sup>, Devanesan Arul Ananth<sup>2</sup> and Thilagar Sivasudha<sup>1\*</sup>

## Abstract

**Background:** Medicinal and aromatic plants have the potential to treat various disorders both human and animals. This present work investigated the hepatoprotective and antioxidant potential of aqueous extract of *Ipomoea staphylina* leaves on Wistar rats against CCl<sub>4</sub>-induced liver injury.

**Methods:** In vitro free radical scavenging activity of aqueous extract of *Ipomoea staphylina* (AIS) was estimated using ABTS [2, 2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)] and DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals. Pharmacologically important phytochemicals such as phenolic compounds and flavonoids were analyzed using HPLC technique. Hepatotoxicity was induced by oral administration of CCl<sub>4</sub> at 1 mL/kg body weight of Wistar rats twice a week for 8 weeks. Experimental rats received AIS orally at the doses of 250 and 500 mg/kg daily for 8 weeks. Silymarin, a routine hepatoprotective drug, was used as a reference drug.

**Results:** Total phenolics and flavonoid content of AIS are 20.78 ± 0.86 mg of GAE/g and 17.78 ± 0.45 mg of RE/g of extract respectively. ABTS and DPPH free radical scavenging activity of AIS exhibits the IC<sub>50</sub> value of 32.08 ± 0.12 and 45.24 ± 0.65 µg/mL respectively. HPLC analysis of AIS showed the presence of polyphenolic compounds such as protocatechuic acid, chlorogenic acid, caffeic acid, vanillin, p-coumaric acid, naringenin, quercetin, rutin and flavon. Treatment with AIS restored the biochemical parameters towards normal level, prevented the occurrence of LPO and significantly ( $P \leq 0.05$ ) increased the levels of enzymatic (SOD and catalase) and non-enzymatic (reduced glutathione) antioxidants in CCl<sub>4</sub> administered Wistar rats. Histopathology results confirmed the hepatoprotective effect of AIS.

**Conclusion:** Based on the results obtained, we suggest that the *I. staphylina* leaves extract have potent antioxidant and hepatoprotective activity.

**Keywords:** Antioxidant, Hepatoprotective, Liver diseases, Medicinal plants

## Introduction

Liver provides protection to an organism against foreign particles by eliminating them, through the process of excretion [1]. Liver damage caused by various hazardous chemicals such as carbon tetrachloride (CCl<sub>4</sub>), thioacetamide, chronic alcohol consumption, and even some drugs used to treat various diseases, may accumulate in the liver tissue and cause alteration in the normal physiological function of the liver by generating free radicals, which is the major reason for the cause of heart related problems like stroke, autoimmune diseases like rheumatoid arthritis, diabetes, and cancer [2]. Patients with liver injury reported to have oxidative stress. The

imbalance in the level of pro-oxidants and antioxidants leads to oxidative stress leads to potential damage for the organism [3]. Currently available synthetic drugs used for the treatment of liver diseases are inadequate and known for various side effects. Earlier scientific studies reported that, the use of plant phenolics, reduces the risk of liver diseases by acting as antioxidants [4]. Recently, interests in consuming edible plants that are rich in antioxidants and health-promoting phytochemicals have been increased worldwide. CCl<sub>4</sub> is frequently used chemical for inducing liver damage in experimental animals [5, 6]. CCl<sub>4</sub> is a potent hepatotoxin, when administered leads to necrosis in liver tissue, ultimately leads to liver injury. CCl<sub>4</sub> induced liver injury depends on metabolism of toxic substance by the liver NAPDH-cytochrome system results in the formation of

\* Correspondence: [sudacoli@yahoo.com](mailto:sudacoli@yahoo.com); [sudha@bdu.ac.in](mailto:sudha@bdu.ac.in)

<sup>1</sup>Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu 620024, India

Full list of author information is available at the end of the article

highly reactive intermediate trichloromethyl free radicals, which binds to cell protein covalently which leads to membrane lipid peroxidation and finally cell necrosis.

India is the land of medicinal plants and it is important to register the data by scientific researches on such plants could be of clinical importance. Treating various health issues like cancer, arthritis, diabetes has been documented in Ayurvedic medical system practiced mainly in the Asian continents for more than 5000 years [7]. *Ipomoea staphylina* Linn. (Convolvulaceae) is widely found in Asian countries including Sri Lanka, India, and China. In Asian traditional medicine system, *I. staphylina* is an extensive climber, commonly used for the treatment of liver diseases, purgation, stomach disorders, pain, inflammation, and in rheumatism [8]. Plants of the same genus such as *Ipomoea hederacea* [9] and *Ipomoea obscura* [10] have been reported for their hepatoprotective activity. Recently, antimicrobial activity of aqueous extract of *Ipomoea reniformis* has been reported [11]. The antioxidant and hepatoprotective activity of *I. staphylina* has not been evaluated scientifically till now. Therefore, identification of phytochemicals from *I. staphylina* aqueous leaves extract (AIS) and evaluate its antioxidant and hepatoprotective activity against  $\text{CCl}_4$  induced oxidative damage in Wistar rats was the main aim of this research.

## Methodology

### Chemicals

All the chemicals used were of great purity and sensible grade. High quality chemicals used in the research were bought from Sigma Chemicals Co. (St. Louis, MO, USA) like  $\text{CCl}_4$ , Folin's reagent,  $\text{Na}_2\text{CO}_3$ ,  $\text{AlCl}_3$ ,  $\text{NaCl}$ ,  $\text{KCl}$ ,  $\text{K}_2\text{S}_2\text{O}_8$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{H}_2\text{O}_2$ , gallic acid, sodium nitrite, rutin,  $\text{C}_2\text{HCl}_3\text{O}_2$ , DPPH, ABTS,  $\text{C}_6\text{N}_6\text{FeK}_3$ , BHT and  $\text{FeCl}_3$ .

### Plant collection and extraction

Plants sample collection and extraction preparations were done by standard protocol followed in Plant metabolite lab, Department of Environmental Biotechnology, Bharathidasan University, Trichirappalli. *I. staphylina* plants samples were collected from local areas near Bharathidasan University, Tiruchirappalli district, Tamilnadu, India and authenticated in the Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India (Collection number 1002, Centre for Research and development of Siddha-Ayurveda Medicines, Bharathidasan University). Leaves were picked, washed, shade dried and ground to powder. Ten grams of the powder was extracted with 250 mL of distilled water (in the ratio 1:25), by proper shaking for 2 days. Whatmann filter paper No.1 was used to filter the extract and followed by lyophilization using a vacuum freeze dryer (Christ alpha 1-2/LD plus, Osterode am Harz, Germany) to produce a yield of 10% of dry extract. The dried

extract was stored at  $-20^\circ\text{C}$  in deep freezer for further study. The extract was dissolved in distilled water of known concentrations and used for experimental studies [11].

### Determination of total phenolic content

Total phenolic content was determined by the standardized protocol [12]. To 100  $\mu\text{L}$  of the AIS (aqueous extract of *I. staphylina*), 1.5 mL of distilled water and 300  $\mu\text{L}$  of Folin-Ciocalteu reagent was added and incubated for 6 min at room temperature. Then, 300  $\mu\text{L}$  of sodium carbonate (2 g/10 mL) was added and allowed to stand for 30 min at  $40^\circ\text{C}$  for colour development. Absorbance was read at 765 nm using the spectrophotometer (Shimadzu UV-1601, Columbia, MD, USA). Commercial gallic acid was used as the standard. Total phenolic content was expressed as mg of GAE/g of extract.

### Determination of total flavonoids content

Total flavonoids content was estimated using the method of Ordon ez et al. [13]. The above mentioned method was standardized in our laboratory to calculate the amount of total flavonoids present in the sample. To 0.5 mL of AIS, 0.5 ml of 2% aluminium chloride solution was added. After 1 h at room temperature, the absorbance was read at 420 nm using a UV-spectrophotometer, and expressed as mg of RE/g of extract. Estimation of total flavonoid in the extracts was carried out in triplicates. Commercial rutin standard was used this assay.

### ABTS free radical scavenging activity

For ABTS assay, the method of Re et al. [14] was adopted with some modifications. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution was prepared first. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark condition. The solution was then diluted by mixing 1 mL ABTS solution with 40 mL of methanol to obtain an absorbance of  $0.706 \pm 0.002$  units at 734 nm using a spectrophotometer. AIS (1 mL) of various concentrations ranging from 20 to 100  $\mu\text{g}$  were allowed to react with 1 mL of the ABTS solution after 7 min the absorbance was read at 734 nm using the spectrophotometer. ABTS scavenging capacity of the extract was compared with that of BHT standard and percentage of inhibition calculated as:

$$\text{ABTS radical scavenging activity (\%)} \\ = \frac{[(\text{Abs control} - \text{Abs sample})]}{(\text{Abs control})} \times 100$$

Where Abs control is the absorbance of ABTS radical + methanol; Abs sample is the absorbance of ABTS radical + sample extract/standard. The sample concentration providing 50% inhibition ( $\text{IC}_{50}$ ) was

calculated from the graph of inhibition percentage against sample concentration. All determinations were carried out in triplicates. ABTS radical-scavenging activity of BHT was also assayed for comparison.

#### DPPH free radical scavenging activity

Effect of extract on DPPH radical was estimated using the method of Liyana-Pathiranan and Shahidi [15]. A solution of 0.135 mM DPPH in methanol was prepared and 1 mL of this solution was mixed with AIS (1 mL) of various concentrations ranging from 20 to 100 µg. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Absorbance of the mixture was read spectrophotometrically at 517 nm. BHT was used as reference standard. The ability to scavenge DPPH radical was calculated by the following equation

$$\text{DPPH radical scavenging activity (\%)} \\ = \frac{[(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100}{}$$

where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard. All determinations were carried out in triplicates.

#### Determination of reducing power activity

Reducing power of the AIS was determined according to the method of Oyaizu [16]. One mL of AIS of various concentrations (20–100 µg) mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then 2.5 mL of 10% trichloroacetic acid was added to the mixture, and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm using a spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power.

#### Polyphenolic compounds identification using HPLC analysis

Identification of polyphenolic compounds from AIS using RP-HPLC with C<sub>18</sub> column (4.6 × 250 mm, 5 µm particle size). HPLC system was coupled with a PDA detector (PDA-2998), Waters, USA. Mobile phase consist of HPLC water with 0.1% formic acid (Solvent A) and 100% methanol (HPLC grade) (Solvent B). Gradient elution program followed; Solvent A and B was 0–10% (5 min), 10–15% (5 min), 15–20% (5 min), 20–30% (5 min), 30–40% (5 min); Flow rate was 1 mL/min and maximum pump pressure 4000 psi was maintained. Polyphenolics were identified at wavelength of 280 nm [17]. Compounds were

identified and confirmed by comparing its tR and λ max compared with commercial standard.

#### In vivo studies

##### Acute toxicity test

To evaluate the acute toxicity of AIS, a single oral dose of AIS of various concentrations 0.5, 1, 1.5, 2, 2.5 and 3.0 g/kg b.w. was given to Wistar rats then allowed free access to food and water. The animals were observed for signs of toxicity such as, urination, salivation, asthenia and defaecation for 72 h after the oral administration for the acute oral toxicity. AIS was safe up to the dose of 2 g/kg b.w. p.o. for Wistar rats and did not show any acute toxicity signs. We chose a wide concentration range for AIS in our preliminary study and their inhibitory effects against CCl<sub>4</sub> induced hepatotoxicity were evaluated (data not shown). By avoiding non effective and toxic concentrations, the 250 and 500 mg/kg b.w. concentrations were selected. Experimental protocols were approved by the CPCSEA, Chennai, Tamil Nadu, India (BDU/IAEC/2012/29).

##### Treatment

Animals were randomly divided into five groups of 6 rats per group. Group I served as normal control and was given olive oil (1 mL/kg of b.w.) daily for a period of 8 weeks. For inducing hepatotoxicity (in vivo), animals of Groups II-V were administered orally 1 mL/kg of b.w. of CCl<sub>4</sub> (1:1, v/v, CCl<sub>4</sub> in olive oil) twice a week for 8 weeks. Group II served as CCl<sub>4</sub>-treated model group (disease control). Group III received silymarin (at 50 mg/kg of b.w.) daily for a period of 8 weeks, which served as positive control. Groups IV and V were administered orally with AIS at 250 and 500 mg/kg of b.w. daily for 8 weeks [18]. At the end of the experiment, all animals were sacrificed. Serum samples were collected into heparinized tubes (50 U/mL). Liver samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible. One part of the liver samples was immediately stored at –20 °C until analysis, another part was excised and fixed in 10% formalin solution for histopathological analysis.

##### Determination of serum biochemical parameters

Serum biochemical parameters such as SGOT, SGPT, ALP and bilirubin were estimated in the serum of experimental animals using commercial reagent kits and were performed according to the manufacturer's (Nice Chemicals [P] Ltd. Cochin, Kerala, India) instructions.

##### Antioxidant enzymes activities

Liver homogenates (10% w/v) prepared in phosphate-buffered saline (PBS containing 137 mM sodium chloride, 2.68 mM potassium chloride, 10.14 mM sodium phosphate

dibasic and 1.76 mM potassium phosphate monobasic in 1000 mL distilled water pH 7.2) were used for antioxidant studies. Catalase activity was determined by the method of Sinha [19] in which dichromatic acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide, with the formation of perchloric acid as an unstable intermediate. The green colour developed was read at 590 nm against blank on a spectrophotometer. CAT activity was expressed as units/mg protein (mmol of hydrogen peroxide consumed/min/mg protein). SOD activity was determined by the method of Marklund and Marklund [20]. The degree of inhibition of pyrogallol auto-oxidation by the supernatant of the tissue homogenate or haemolysate was measured. The change in absorbance was read at 470 nm against blank every minute for 3 min using a spectrophotometer. Enzyme activity was expressed as U/mg of protein. GSH content was estimated by the method of Moron et al. [21]. To the supernatant of the tissue homogenate, 0.5 mL of 10% trichloroacetic acid was added followed by centrifugation. To the resulting protein-free supernatant, 4 mL of 0.3 M sodium phosphate dibasic (pH 8.0) and 0.5 mL of 0.04% (w/v) 5, 5-dithiobis-2-nitrobenzoic acid were added. Absorbance of the resulting yellow colour was read spectrophotometrically at 412 nm. Results were expressed as  $\mu\text{g}/\text{mg}$  protein.

#### Determination of lipid peroxidation level

The mean concentration of MDA, a measure of the intensity of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa et al. [22]. Briefly, to 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.81% thiobarbituric acid aqueous solution were added in succession. To this reaction, 0.2 mL of tissue homogenate was added separately. This mixture was then heated in a boiling water bath for 60 min. After cooling to room temperature, 5 mL of butanol:pyridine (15:1, v/v) solution was added. The mixture was then centrifuged at 2000 x g for 15 min. Upper organic layer was separated and the intensity of the resulting pink color was read at 532 nm. Tetramethoxypropane was used as an external standard. The level of lipid peroxides was expressed as nanomoles of MDA formed/mg protein.

#### Histological studies

Liver samples were fixed in Bouin's fixative and processed to obtain 5  $\mu\text{m}$  thick paraffin sections and stained with hematoxylin and eosine (H&E) for histological observations.

#### Statistical analysis

Statistical analysis was performed using the statistical package SPSS (Statistical Package for Social Science,

SPSS Inc., Chicago, IL, USA). Analysis of variance was performed by ANOVA procedures and significance of each group was verified with one-way analysis of variance followed by Duncan post hoc test ( $P \leq 0.05$ ). Values obtained are means of six replicate determinations  $\pm$  standard deviation.

## Results

#### Preliminary phytochemical analysis

Preliminary phytochemical analysis [18] of AIS revealed the presence of phenolics, flavonoids, steroids, tannins, saponins and terpenoids. Total phenolics and flavonoid content of AIS were  $20.78 \pm 0.86$  mg of GAE/g of the extract and  $17.78 \pm 0.45$  mg of RE/g of extract respectively.

#### Free radical scavenging activity

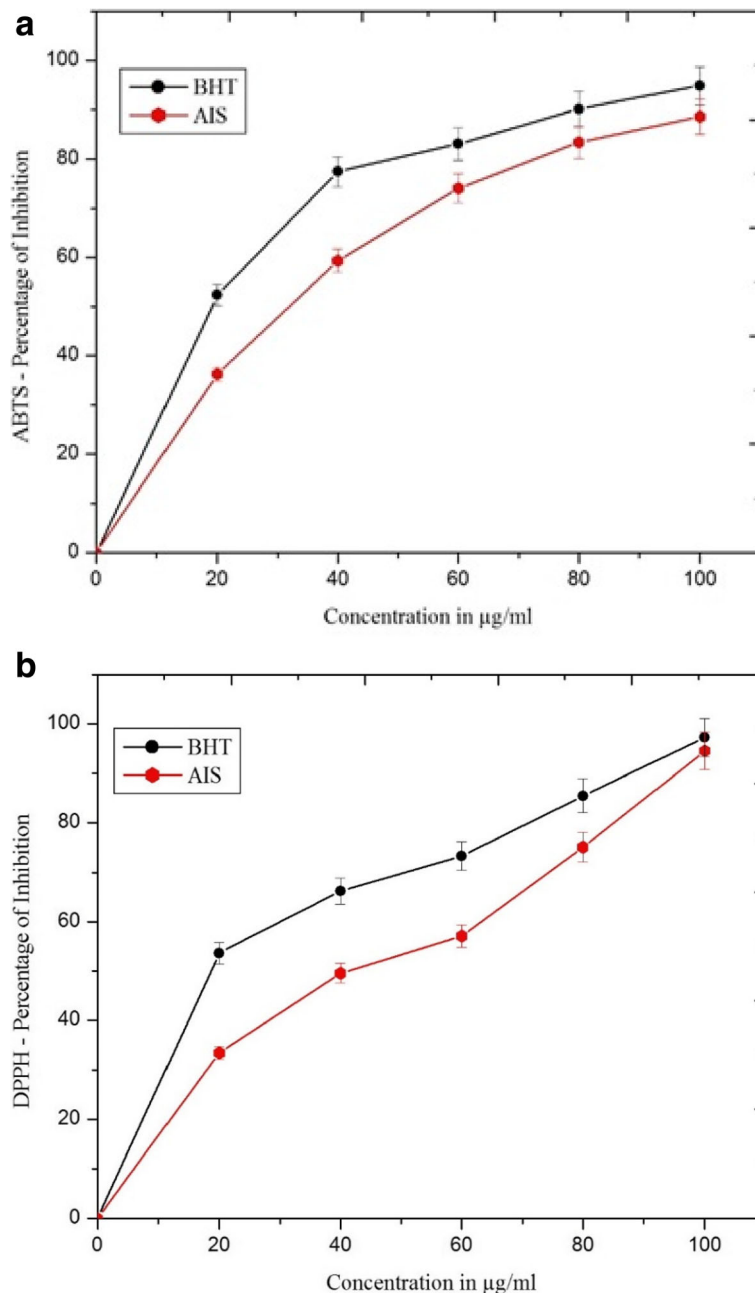
Inhibition range of ABTS (Fig. 1a) and DPPH (Fig. 1b) free radicals by AIS are shown in Figures. The quenching effect of AIS against ABTS and DPPH radicals in a dose dependent manner is clearly depicted in the result obtained.  $\text{IC}_{50}$  values of AIS to quench ABTS and DPPH radicals are  $32.08 \pm 0.12$  and  $45.24 \pm 0.65$   $\mu\text{g}/\text{mL}$ , respectively.

#### Reducing power activity

The reducing potential of the AIS was shown in Fig. 2 in comparison with a standard BHT at 700 nm. In the reducing power assay, the principle is the ability of the antioxidants present in the sample to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by donating an electron and results in the formation of perl's blue coloured solution. The intensity of the colour formed can be measured at 700 nm. The reducing effect of the extract can be predicted by increase in absorbance in dose dependent manner. The reducing capacity of AIS increases proportionally with increase in the concentration of the extract used. The results shows that the reducing at 100  $\mu\text{g}/\text{mL}$  concentration had an optical density of 0.4274, which was comparatively similar to that of the standard BHT with an optical density of 0.4766.

#### HPLC identification of polyphenolic compounds

A typical HPLC profile of the phenolic and flavonoids constituents of AIS was presented in Fig. 3. HPLC analysis of AIS showed that phenolic compounds such as protocatechuic acid, chlorogenic acid, caffeic acid, vanillin and p-Coumaric acid and also flavonoids such as naringenin, quercetin, rutin and flavon were detected (Fig. 3c). Flavonoids are the most abundant phytochemicals present in the AIS as compared with phenolic compounds. Chromatograms revealed some unidentified peaks that may correspond to unknown phenolic compounds.



**Fig. 1 a** Antioxidant activity of AIS. **a** ABTS<sup>+</sup> radical scavenging activity of AIS compared with BHT. Each value is expressed as the mean ± standard deviation (n = 3). **b** DPPH radical scavenging activity of AIS compared BHT. Each value is expressed as the mean ± standard deviation (n = 3)

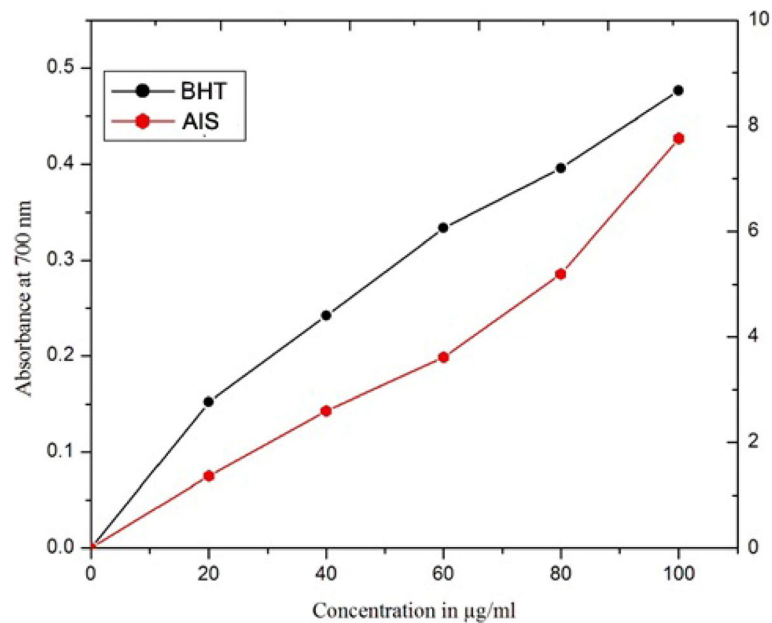
**In vivo studies**

**Serum biochemical parameters**

Mean serum activities of SGOT, SGPT, ALP, and bilirubin were found to be higher in samples from Group II rats (Disease control) when compared to values in samples from Group I (Normal control) rats. However, the mean serum activities of above mentioned parameters were significantly ( $P \leq 0.05$ ) lower in groups treated with AIS (500 mg/kg b.w) and silymarin (Table 1).

**Antioxidant enzyme activities**

CCl<sub>4</sub> treatment caused a significant decrease in the level of tissue antioxidant enzymes such as SOD, CAT, and the cellular antioxidant molecule GSH in liver [23]. AIS administration has up regulated the activities of these endogenous antioxidant enzymes (Table 1). The levels of liver SOD, CAT, and GSH in the CCl<sub>4</sub>-treated group were less compared with the normal control group. The result obtained indicates the tremendous increase ( $P \leq 0.05$ ) in



**Fig. 2** Reducing power of AIS compared with BHT. Each value is expressed as the mean  $\pm$  standard deviation ( $n = 3$ )

SOD, CAT and GSH activity in the AIS treated groups (at 500 mg/kg b.w.). The activity of AIS at the dose of 500 mg/kg was comparable to that of the positive control, silymarin. Reduced glutathione is presumed to be an important endogenous defense against peroxidative destruction of cellular membranes. In the present study,  $\text{CCl}_4$  administrated animals shows decline in the presence of reduced glutathione level. Treatment with AIS was very effective in restoring the glutathione content which had been substantially decreased in  $\text{CCl}_4$  group (Table 1). Significantly ( $P \leq 0.05$ ) higher MDA level was noted in tissue samples obtained from  $\text{CCl}_4$  administrated (Group II) rats than in those from normal (Group I) rats, whereas the mean levels of MDA in samples from AIS-treated groups (Group IV and Group V) were significantly ( $P \leq 0.05$ ) lower than the levels in samples from (Group II) rats given in Table 1.

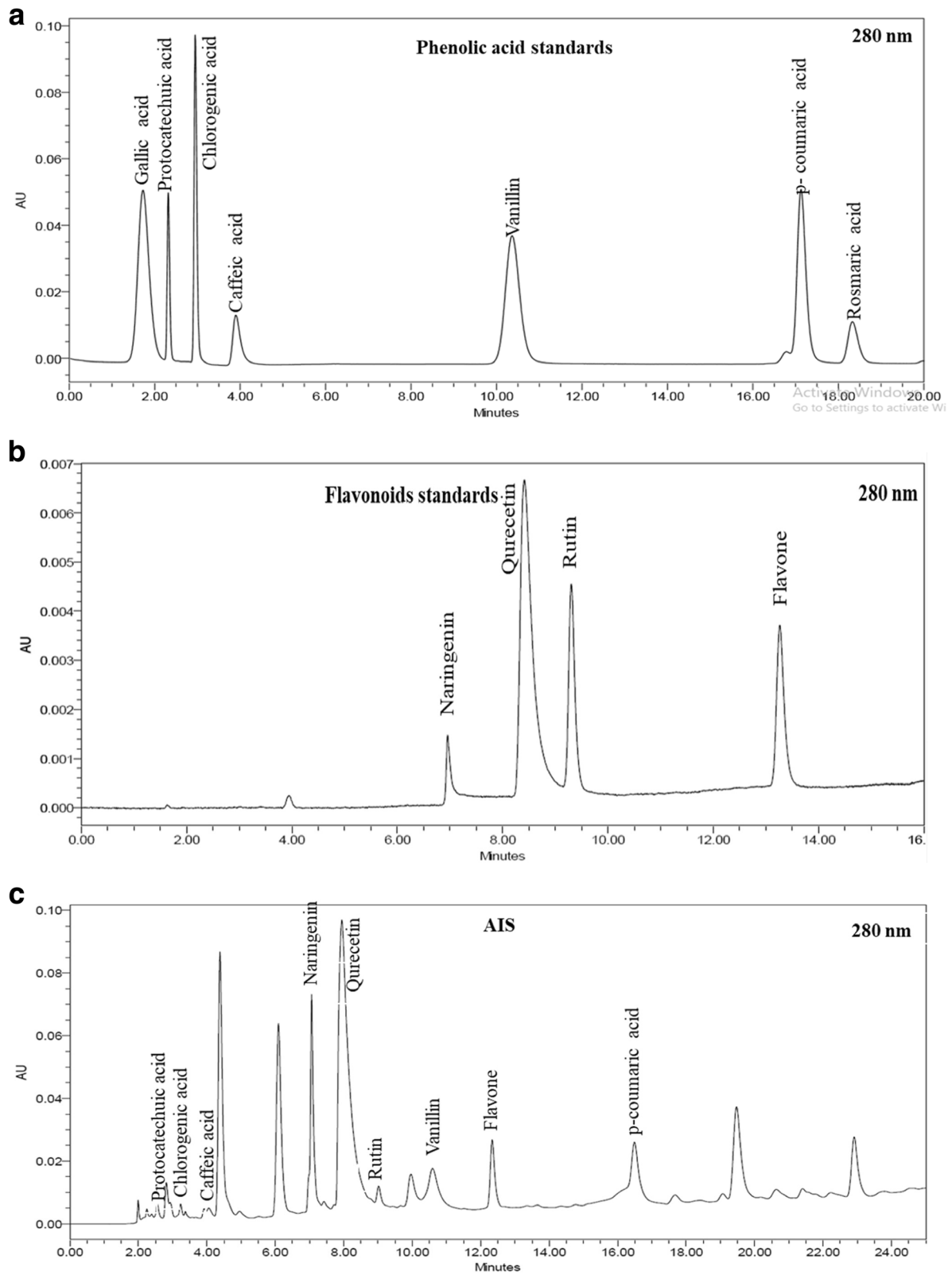
#### Histopathological examination

The report of histopathological studies supports the results of the biochemical studies and it demonstrates that the hepatic damage caused by  $\text{CCl}_4$  during intoxication is reduced in the liver sample of animals treated with AIS. Histological examination of the liver sections of control animals revealed the presence of normal hepatocytes with well-preserved cytoplasm, a prominent nucleus and distinct sinusoidal spaces (Fig. 4a).  $\text{CCl}_4$  treated groups liver tissue cells show severe damage to liver tissue by cell necrosis (Fig. 4b). Interestingly, the administration of AIS against this liver damage shows

protective capacity of AIS against liver necrosis. AIS at a dose of 500 mg/kg b.w. was more effective when compared to the other dose (250 mg/kg bw) (Fig. 4c) and also showed regaining of normal histological appearance (Fig. 4d), which clearly indicates the protective and curative effect of AIS against  $\text{CCl}_4$ -induced hepatic damage. The standard, silymarin-treated rats liver section showed almost normal liver with no sign of necrosis (Fig. 4e).

#### Discussion

Phenolic compounds acting as antioxidants present in the plant products are gaining attention among different people due to their health benefits. In this study, antioxidant assays like ABTS, DPPH and reducing power assays have been carried to explore the antioxidant activity of AIS. These assays are the mostly used common assays by researchers to evaluate the antioxidant capacity of samples from plants. In the present study, scavenging activity of AIS on DPPH and ABTS radicals were observed in a concentration-dependent pattern. The antioxidant ability of the secondary metabolites of plants is also related to its reducing capacity [24]. As already discussed above, the reducing capacity of a substance is based on the reduction of the complex compound ferric tripyridyl triazine ( $\text{Fe}^{3+}$ -TPTZ) to ferrous tripyridyl triazine ( $\text{Fe}^{2+}$ -TPTZ), which results in the formation of coloured solution. Reducing power efficacy of the AIS increases in a concentration dependant manner. Due to the chemical complexity of plant extract, the isolation and quantification of polyphenolic compounds are very difficult. HPLC is an important analytical method in



**Fig. 3** HPLC profiling of polyphenolic compounds identified in AIS

**Table 1** Protective effect of AIS on CCL<sub>4</sub> induced elevation in hepatic enzymes, Bilirubin, TBARS content, and depletion of enzymatic and non enzymatic enzymes in Wistar rats

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	TBARS <sup>a</sup>	GSH <sup>b</sup>	SOD <sup>c</sup>	CAT <sup>d</sup>
Group 1 (Normal Control)	52.42 ± 1.87 <sup>d</sup>	36.65 ± 0.61 <sup>d</sup>	90.86 ± 1.21 <sup>c</sup>	0.67 ± 0.08 <sup>c</sup>	6.02 ± 0.71 <sup>c</sup>	24.60 ± 1.14 <sup>a</sup>	23.00 ± 1.58 <sup>a</sup>	57.20 ± 2.58 <sup>a</sup>
Group 2 (Disease Control)	156.97 ± 1.84 <sup>a</sup>	117.04 ± 6.21 <sup>a</sup>	278.48 ± 0.74 <sup>a</sup>	1.45 ± 0.11 <sup>a</sup>	14.63 ± 1.08 <sup>a</sup>	8.00 ± 1.58 <sup>c</sup>	8.40 ± 1.14 <sup>d</sup>	37.00 ± 1.58 <sup>c</sup>
Group 3 (Silymarin)	59.50 ± 1.12 <sup>c</sup>	46.54 ± 4.58b <sup>c</sup>	90.60 ± 4.27 <sup>c</sup>	0.69 ± 0.06 <sup>c</sup>	6.64 ± 0.33 <sup>c</sup>	23.20 ± 3.03 <sup>a</sup>	18.60 ± 1.14 <sup>b</sup>	59.00 ± 1.58 <sup>a</sup>
Group 4 (AIS 250 mg/kg b.w)	64.53 ± 5.60 <sup>b</sup>	51.46 ± 7.56 <sup>b</sup>	111.00 ± 9.05 <sup>b</sup>	0.80 ± 0.02 <sup>b</sup>	8.40 ± 1.14 <sup>b</sup>	16.20 ± 1.92 <sup>b</sup>	15.80 ± 1.30 <sup>c</sup>	46.00 ± 4.30 <sup>b</sup>
Group 5 (AIS 500 mg/kg b.w)	58.11 ± 0.77 <sup>c</sup>	44.20 ± 4.43 <sup>c</sup>	89.50 ± 8.66 <sup>c</sup>	0.71 ± 0.05 <sup>bc</sup>	5.68 ± 0.85 <sup>c</sup>	24.80 ± 1.92 <sup>ab</sup>	23.00 ± 2.23 <sup>a</sup>	57.80 ± 2.77 <sup>a</sup>

Data are expressed as mean ± SD (n = 6)

Mean values with different superscripts are significantly different from each other as revealed by Duncan post hoc test (P ≤ 0.05)

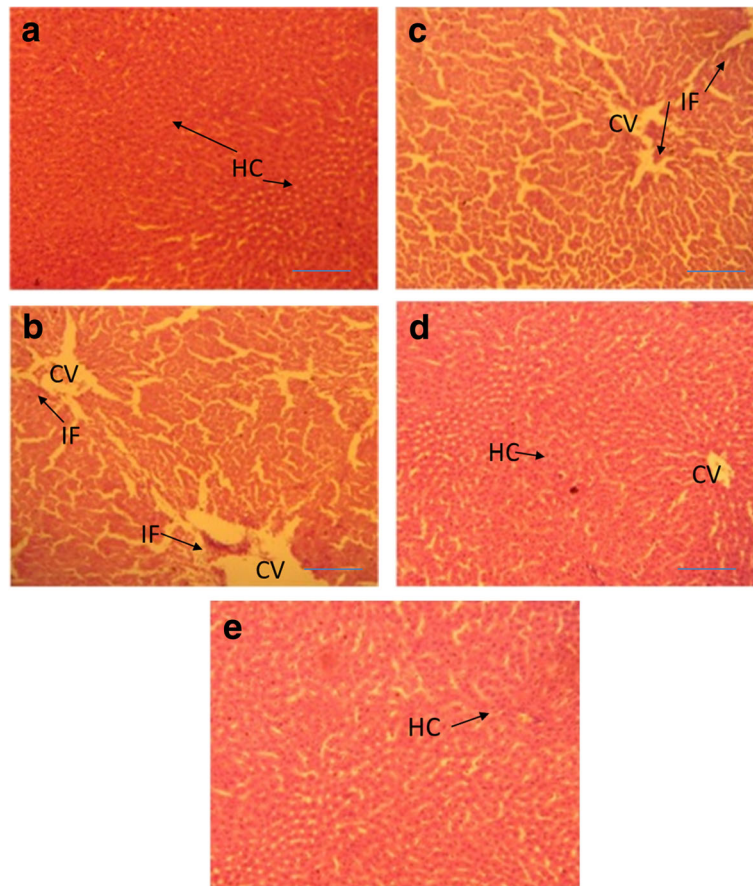
<sup>a</sup>mmol/mg protein

<sup>b</sup>μg of reduced glutathione / mg protein

<sup>c</sup>U/mg of tissue

<sup>d</sup>μmol of H<sub>2</sub>O<sub>2</sub> utilized/min/mg of protein





**Fig. 4** Histopathological analysis of liver tissue of male Wistar rats administrated with  $\text{CCl}_4$  and treated with AIS. **a** Control group shown normal lobular architecture with clear central vein (X-40). **b**  $\text{CCl}_4$  administered group shown severe hepatocyte necrosis, fatty degeneration and vacuolation (X-40). **c** AIS treated group at 250 mg/kg shown mild improvement in chord arrangement but perinuclear vacuolation was visible (X-40). **d** AIS treated group at 500 mg/kg shown normal cellular architecture with distinct hepatic cells sinusoidal spaces (X-40). **e** Silymarin (positive drug) treated group exhibited almost normal histology (X-40); Hepatocytes (HC), Central vein (CV) and Cellular inflammation (IN)

identification of bioactive compounds from plant sources. In this study we identified important bioactive compounds using HPLC. These phytochemicals have effective antioxidant potential to treat various human diseases [25]. Antioxidant properties of bioactive compounds (phenolics and flavonoids) are responsible for the hepatoprotection by plant extracts. These compounds effectively reduce the oxidative stress and other inflammatory responses [26, 27].

Protective effect of AIS was further evaluated in  $\text{CCl}_4$ -induced oxidative stress in the livers of Wistar rats by observing the histological and biochemical changes in rats. The liver injury caused by  $\text{CCl}_4$  is mainly by the release of free radicals, which are generated during the metabolism of  $\text{CCl}_4$  by the cytochrome P450 (CYP) system. The content of CYP in liver is more abundant than that in other organs such as lung, kidney, and intestine. By the activation of liver CYP, by  $\text{CCl}_4$  intoxication produces hepatotoxic metabolite trichloromethyl free radicals ( $\bullet\text{CCl}_3$  or  $\text{CCl}_3\text{OO}\bullet$ ), which immediately propagate a chain of

lipid peroxidation events and ultimately leads to the breakdown of membrane structure and result in the leakage of hepatic cell marker enzymes into the blood. In our study, significant increase ( $P \leq 0.05$ ) in the levels of hepatic enzymes such as SGOT, SGPT, ALP and bilirubin were observed in serum sample of experimental animals after administration of  $\text{CCl}_4$ . The increased levels of liver enzymes in blood of rats administrated with  $\text{CCl}_4$  were reduced by the treatment with AIS. The degree of liver cell membrane damage is known clinically by the increased activity of SGOT, SGPT, and ALP enzyme in serum. The hepatic TBARS level is also commonly used as an indicator of liver tissue damage which involves a series of chain reactions [28]. In our results, rats treated with  $\text{CCl}_4$  showed an increase in TBARS which was restored towards normal by the treatment with AIS.

Antioxidant enzyme such as SOD converts superoxide anion into  $\text{H}_2\text{O}_2$  and  $\text{O}_2$ , whereas CAT reduces  $\text{H}_2\text{O}_2$  to  $\text{H}_2\text{O}$ , resulting in the quenching of free radicals. SOD

and CAT plays an important role in the elimination of ROS derived from the redox process in liver tissues and considered to be most important enzymes [29]. Intoxication with  $\text{CCl}_4$  to Wistar rats reduced the antioxidant capacity of rat liver by inhibiting the enzymatic activity of SOD and CAT. However, treatment with AIS improved the levels of these enzymes significantly in a dose dependent manner (250 and 500 mg/kg b.w). The activity of AIS was directly proportional to the increased concentration, suggesting that it could offer protection against hepatic damage caused by toxic substances.  $\text{CCl}_4$  administration reduces the level of GSH in liver tissue of rats. Administration of AIS as a drug to treat hepatic damage significantly increased the GSH content in liver tissue ( $P \leq 0.05$ ). Similarly hydroalcoholic extract of *I. staphylinina* leaves significantly decreased the serum urea, creatinine, uric acid and protein levels in treated groups, which strongly supports our data [30]. In support with the results of biochemical parameters, treatment with AIS reduced the histological alteration induced by  $\text{CCl}_4$ .

## Conclusion

Our study demonstrated that the AIS leaves showed favorable potency to protect the liver damage induced by  $\text{CCl}_4$ , which suggested that *I. staphylinina* can be used as a potential hepatoprotective drug. The hepatoprotective activity of AIS against severe damage caused by  $\text{CCl}_4$  intoxication was clearly proved by the results obtained. This is mainly achieved by its ability to neutralize the free radicals. Simultaneously inflammatory responses were suppressed and improved activity of drug-metabolizing enzyme is noted well from the results obtained. HPLC results revealed that *I. staphylinina* extract have potential bioactive secondary metabolites. Future studies are in progress to better understand the mechanism of action of bioactive compounds in AIS responsible for its hepatoprotective and antioxidant effects.

## Abbreviations

%: percentage; °C: degree Celsius; ABTS: 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); AIS: *Ipomoea staphylinina* leaves aqueous extract; ALP: Alkaline phosphatase; b.w.: body weight; BHT: Butylated hydroxytoluene; CAT: Catalase;  $\text{CCl}_4$ : Carbon tetrachloride; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; GAE: Gallic acid equivalent; GSH: Glutathione; h: hours; Kg: kilogram; LPO: Lipid peroxidation; M: Molar; MDA: Malondialdehyde; mg: milligram; min: minutes; mL: milliliter; mM & mmol: millimolar; NADPH: Nicotinamide adenine dinucleotide phosphate; nm: nanometer; PDA: Photo Diode Array; psi: Pounds per square inch; RE: Rutin equivalent; ROS: Reactive oxygen species; RP-HPLC: Reverse Phase High Performance Liquid Chromatography; rpm: rotation per minute; SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reacting substances; tR: Retention time; U: units; v/

v: volume/volume;  $\lambda$  max: Absorbance spectrum;  $\mu\text{g}$ : microgram;  $\mu\text{L}$ : microliter;  $\mu\text{mol}$ : micromolar

## Acknowledgements

We thank Dr. J. Senthilkumar, Veterinary Surgeon and Dr. R. Banumathi for their assistance in interpretation of histological results. Finally we sincerely thank UGC and DST-FIST, India for providing instrument facility.

## Funding

No funding.

## Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

## Authors' contributions

RJ & Dr. DAA have conducted all the experiments. TS has edited the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Experimental procedures and protocols were approved by the committee for the purpose of Control and supervision of experiments on animals, Chennai, Tamil Nadu, India.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## Author details

<sup>1</sup>Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu 620024, India. <sup>2</sup>Department of Food Quality & Safety, Gilat Research Center, Agricultural Research Organization, Beer Sheva 85280, Israel.

Received: 2 November 2018 Accepted: 7 April 2019

Published online: 26 April 2019

## References

- Shaker ME, Zalata KR, Mehal WZ, Shiha GE, Ibrahim TM. Comparison of imatinib, nilotinib and silymarin in the treatment of carbon tetrachloride-induced hepatic oxidative stress, injury and fibrosis. *Toxicol Appl Pharmacol*. 2011;252:165–75.
- Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press; 1989. p. 96–8.
- Halliwell B, Gutteridge JM. 1990. Role of free radicals and catalytic metal ions in human diseases: an overview. *Methods Enzymol* 1990;186:1–8.
- Gaur G, Alam MS, Jabbar Z, Javed K, Athar M. Evaluation of antioxidant activity of *Cassia siamea* flowers. *J Ethnopharmacol*. 2006;108:340–8.
- Ugur H, Amat N, Blazekovic B, Talip A. Protective effect of *Cichorium glandulosum* root extract on carbon tetrachloride-induced and galactosamine-induced hepatotoxicity in mice. *Food Chem Toxicol*. 2009;47:2022–30.
- Saleem TSM, Chetty CM, Ramkanth S, Rajan VST, Kumar KM Gauthaman K. Hepatoprotective herbs – a review. *Int J Biol Pharm Allied Sci*. 2010;1:1–5.
- Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. *Ind J Pharmacol*. 2000;32:S81–S118.
- Mullaicharam AR, Maheswari RU, Geetha K, Panicker PS, Chandralekha V. Hemorrhoids: a review. *Res J Pharm Technol*. 2010;3:296–9.
- Devi RS, Chitra M, Jayamathi P. Hepatoprotectivity and an antioxidant study of *Ipomoea hederacea* on experimentally induced hepatotoxic rats. *Recent Res Sci Technol*. 2010;2:17–9.
- Mungole AJ, Awati R, Chaturvedi A, Zanwar P. Preliminary phytochemical screening of *Ipomoea obscura* (L) - a hepatoprotective medicinal plant. *Int J Pharm Tech Res*. 2010;2:2307–12.

11. Rameshkumar A, Sivasudha T, Jeyadevi R, Sangeetha B, Ananth DA, GSB A, Nagarajan N, Renganathan R, Kathiravan A. In vitro antioxidant and antimicrobial activities of *Merremia emarginata* using thio glycolic acid-capped cadmium telluride quantum dots. *Colloids Surf B Biointerfaces*. 2013;101:74–82.
12. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. *J Agri Food Chem*. 2003;51:609–14.
13. Ordonez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Saisium edule* (Jacq.) swart extracts. *Food Chem*. 2006;97:452–8.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999;26:1231–7.
15. Liyana-Pathiranan CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J Agri Food Chem*. 2005;53:2433–40.
16. Oyaizu M. Studies on products of the browning reaction antioxidative activities of browning reaction products prepared from glucosamine. *JapJ Nutr Diabet*. 1986;44:307–15.
17. Rameshkumar A, Sivasudha T, Jeyadevi R, Ananth DA, Pratheepa G. Effect of environmental factors [air and UV-C irradiation] on some fresh fruit juices. *European Food Research and Technology*. 2012;234:1063–70.
18. Jeyadevi R, Sivasudha T, Rameshkumar A, Harnly JM, Lin LZ. Phenolic profiling by UPLC–MS/MS and hepatoprotective activity of *Cardiospermum halicacabum* against CCl<sub>4</sub> induced liver injury in Wistar rats. *J Funct Food*. 2013;5:289–98.
19. Sinha AK. Colorimetric assay of catalase. *Anal Biochem*. 1972;47:389–94.
20. Marklund S, Marklund G. Involvement of the superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay for superoxide dismutase. *Biochim Biophys Acta*. 1974;47:469–74.
21. Moron MS, Depierre JN, Mannervik V. Levels of glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta*. 1979;582:67–8.
22. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem*. 1979;95:351–8.
23. Sheweita SA, El-Gabar MA, Bastawy M. Carbon tetrachloride changes the activity of cytochrome P450 system in the liver of male rats: role of antioxidants. *Toxicol*. 2001;169:83–92.
24. Orhan I, Kartal M, Abu-Asaker M, Senol FS, Yilmaz G, Sener B. Free radical scavenging properties and phenolic characterization of some edible plants. *Food Chem*. 2009;114:276–81.
25. Ananth DA, Tietel Z, Aseervatham GSB, Deviram G, Sivasudha T. Phytochemical and pharmacological status of indigenous medicinal plant *Pedaliium murex* L. *Biomed Pharmacother*. 2018;103:1456–63.
26. Arif M, Fareed S, Rahman MA. Stress relaxant and antioxidant activities of acid glycoside from *Spondias mangifera* fruit against physically and chemically challenged albino mice. *J Pharm Bioall Sci*. 2016;8:58–63.
27. Iqbal SS, Mujahid M, Kashif SM, Khalid M, Badruddeen AM, Bagga P, Akhtar J, Rahman MA. Protection of hepatotoxicity using *Spondias pinnata* by prevention of ethanol-induced oxidative stress, DNA-damage and altered biochemical markers in Wistar rats. *Integr Med Res*. 2016;5:267–75.
28. Poli G. 1993. Liver-damage due to free-radicals. *Br Med Bull* 1993;49:604–620.
29. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. *Cell Mol Life Sci*. 2004;61:192–208.
30. Bag AK, Mumtaz SMF. Hepatoprotective and nephroprotective activity of hydroalcoholic extract of *Ipomoea staphylina* leaves. *Bangladesh J Pharmacol*. 2013;8:263–8.

Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

---

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)

---