

# IN VIVO AND *in VITRO* ANALYSES to REVEAL THE POTENTIAL of SOLANUM VIOLACEUM as EFFICIENT HEPATOPROTECTIVE AGENT

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## **Abstract:**

### **Objectives**

The antioxidant and hepatoprotective activity of *Solanum violaceum* was evaluated with carbon tetrachloride (CCl<sub>4</sub>) intoxicated animal models followed by phytochemical screening of bioactive extracts by GC-MS.

### **Methods**

The total phenolic and flavonoid content were estimated from the solvent extracted samples. *In vitro* antioxidant and *in vivo* hepatoprotective studies were carried out with the ethyl acetate extract of *Solanum violaceum* (SVEE). The hepatoprotective evaluations were carried out with CCl<sub>4</sub> intoxicated rats. The levels of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), bilirubin and proteins were measured in the serum and histopathological studies were carried out with the liver sections. The phytochemical profile of the bioactive extract was revealed from GC-MS analysis.

### **Results**

The ethyl acetate and chloroform extracts of *Solanum violaceum* were rich in phenolic and flavonoid contents respectively. The DPPH and NO scavenging assays revealed better activity for ethyl acetate extract while ABTS assay was better for the alcoholic extract. Therefore further investigations on the hepatoprotective activity were carried out with ethyl acetate extract. The hepatoprotective screening revealed the restoration of enzymes as well as protein to normal levels upon treating intoxicated rats with the bioactive extracts in a dose dependant manner. Further histopathological studies also revealed that the ethyl acetate extract was effective towards restoration of liver cells to normal levels. The phytochemical profile as evaluated from the GC-MS analysis of the extract narrowed down the observed response to four major compounds.

### **Conclusions**

*Solanum violaceum* possesses significant antioxidant and hepatoprotective activities. The bioactive constituents attributing to the observed activity were revealed by GC-MS analysis. Viridiflorol, palmitic acid, n-pentacosanal and citroflex A were found to be the major ones.

**Keywords:** *Solanum violaceum*, *Solanum indicum*, Hepatoprotective activity, DPPH, CCl<sub>4</sub>, ABTS, NO, GC-MS.

## INTRODUCTION

The diverse chemical and biological properties of the genus *Solanum* have opened avenues in natural drug discovery for more than 3 decades. Around 2000 species have been reported in the genus and is considered as one of the largest genus in the Solanaceae family. Their diverse pharmacological actions include anti-inflammatory, antiviral, antifungal, antiasthmatic, antioxidant, antitumor, anticonvulsant, cardiovascular, antihypertensive, hepatoprotective, anticancer, antimicrobial, antianaemic, antihistaminic, diuretic, hypolipidemic, mosquito repellent, anthelmintic, spasmolytic, vasorelaxant, antiurolithiatic, antinociceptive, antidepressant, analgesic, expectorant, laxative etc. Among the 670 chemical constituents reported in the literature, the important classes are steroidal saponins, fatty acids and their esters, pregnanes, lignans, flavonoids, steroidal alkaloids, coumarins and sterols<sup>1</sup>.

*Solanum violaceum* (synonym *S.indicum*), a plant belonging to genus *Solanum* has received great importance in alternative system of medicines. It is an important ingredient in various ayurvedic formulations like dasamoolarishta<sup>2</sup>. *S. violaceum* is traditionally used for relieving vomiting, abdominal pain, cough, ulcer related pain, dyspepsia and skin diseases<sup>3</sup>. Various parts of the plants like root, fruit, seeds etc. have diverse traditional uses. These include carminative, analgesic, laxative, anthelmintic, aphrodisiac, astringent, expectorant, anthelmintic, cardiogenic etc<sup>4</sup>. Moreover, *S.violaceum* was reported to have anti-inflammatory, antipyretic, antimicrobial, anticancer, anthelmintic, wound healing, cytotoxicity, hypolipidemic, thrombolytic and antifungal activity<sup>5</sup>. The isolated constituents from the extracts include indiosides G-O (steroidal saponins), spirostanesaponins, diosgenin, yamogenin, yamogenone, borassosides, zingiberosides, tricalysioside, daucosterol, oxositosterol, stigmasterol and ergosterol derivatives<sup>1</sup>.

Plants with huge amount of phenolics, flavonoids, proanthocyanidins, sterols etc. have great importance in life systems for their powerful antioxidant property<sup>1</sup>. Antioxidants delay auto oxidation by scavenging the free radicals generated within the body. The most common free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS cause oxidative damage associated with aging, liver disorders, heart diseases, cancer and membrane damage<sup>6</sup>. The most functional antioxidants interfere with the free radical chain reaction by donating H<sup>+</sup> ions and destroy the chain process<sup>7</sup>. For the past few decades, antioxidants have got their clinical importance in the prevention of various diseases. High toxicity, instability, and volatility of synthetic phenolic compounds like butylated hydroxyl anisole, butylated hydroxyl toluene, tertiary butyl hydroxyl quinone have limited their use as antioxidants. Hence, there has always been a need for better alternatives from natural sources with minimal side effects. In addition to this, practical evidences suggest the use of natural antioxidants to treat hepatic diseases<sup>8</sup>.

Hepatic diseases cause damage to the cells, tissues, structure or functions of the liver. The damage can further be induced by several other factors including biological, chemical, autoimmune disorders, etc<sup>9</sup>.

Carbon tetrachloride (CCl<sub>4</sub>) is a well-known mediator for hepatotoxin that is used to induce hepatotoxicity in animal-models. It is metabolized by microsomal cytochrome (CYP) P450 enzymes into highly reactive metabolites as trichloromethyl (-CCl<sub>3</sub>) and trichloromethyl peroxy (-CCl<sub>3</sub>OO) radicals. It further activates Kupffer cells to initiate the hepatic inflammation process where proinflammatory mediators such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and interleukin-6 (IL-6) are generated and lipid peroxidation gets induced. CCl<sub>4</sub> model in rodents has been extensively used to evaluate the efficacy of

hepatoprotective drugs as they reflect the metabolic and morphological changes during liver injury with highly reproducibility<sup>10</sup>.

Based on the aforesaid therapeutic values of the genus, *Solanum* and the limited availability of literature reports on evaluation of hepatoprotective activity of *S.violaceum*, the present study was carried out to investigate further on the antioxidant potential and hepatoprotective activity, along with phytochemical profiling of the bioactive extract.

## MATERIALS AND METHODS

### Collection and authentication

Aerial parts of *Solanum violaceum* were collected from Malappuram, Kerala during June to August. The plant was identified by Kottakkal Ayurveda research Centre, Malappuram, Kerala as *Solanum violaceum* Ortega and a voucher specimen was deposited for further references<sup>11, 12</sup>.

### Preparation of extracts

Around 600 g of the aerial parts of *S.violaceum* were dried under shade. Successive solvent extraction was carried out using petroleum ether, chloroform, ethyl acetate, alcohol and water. Each extract was concentrated in a rotary evaporator and stored at 4 °C until further use.

### Phytochemical screening

#### *Qualitative analysis of phytochemicals*

Preliminary phytochemical screening was carried out for each extracts as per standard procedure. The extracts were analyzed for carbohydrates, proteins, steroids, volatile oils, glycosides, flavonoids, alkaloids, tannins and phenolics<sup>11, 12</sup>.

#### *Quantitative analysis of phytochemicals*

#### Estimation of total polyphenol

Folin-ciocalteu's (FC) colorimetric method was used for the estimation of total phenolic content in all the extracts. One ml of each extract was mixed with one ml of FC reagent, which was diluted to 10 fold with distilled water prior to use. After three minutes, the solution was mixed with 3 ml of 2 % sodium carbonate solution. Whole solution was mixed well and was incubated for two hours at room temperature. The total polyphenol content was calculated from the absorbance values at 725 nm<sup>13, 14</sup>.

#### *Total flavonoid content*

The total flavonoid content of each extract was estimated by aluminium chloride ( $\text{AlCl}_3$ ) colorimetric method as described in the literature with some modifications. Each sample (0.5ml) was mixed with 2 ml water and 0.15 ml 15 %  $\text{NaNO}_2$  solution. After 6 minutes, 0.15 ml of 6 %  $\text{AlCl}_3$  was added. Again after 6 minutes, 2 ml of 4 % sodium hydroxide solution was added. Volume was increased up to 5 ml with distilled water. After 15 minutes, absorbance measured at 510 nm was used to estimate the total flavonoid content<sup>15-17</sup>.

## Invitro antioxidant study

### *DPPH Method*

3.0 ml of DPPH solution (50 µM) in methanol was added to 100 µl of varying concentrations of each extract. Ascorbic acid was used as standard. The sample prepared without the extract was used as a negative control. The reaction mixture was incubated in dark at room temperature for 20 minutes. Then the absorbance was measured at 515 nm. The inhibition potential was evaluated using the following equation<sup>18</sup>.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \quad (\text{Equation 1})$$

### *Nitric oxide radical scavenging*

1.5 ml of 5 mM sodium nitroprusside solution in phosphate buffer (pH 7.4) was mixed with different concentrations of the plant extracts. The resulting solution was incubated at 25°C for 3 hours. The nitric oxide scavenging activity was determined by using Griess reagent (1 % sulphanilamide, 2 % phosphoric acid and 0.1 % N-1-naphthyl ethylene diamine dihydrochloride). After the incubation time, the solution was diluted with 1.5 ml of Griess reagent. Ascorbic acid was used as a standard. The diazotization reaction of the nitrate with sulphanilamide and its further coupling with N-1 naphthyl ethylene diamine dihydrochloride results in a chromophore whose absorbance was measured at 546 nm. The percentage scavenging activity was measured using the equation 1<sup>19, 20</sup>.

### *ABTS method*

The reaction mixture containing 7 mM of aqueous ABTS and 2.45 mM Potassium per sulfate was kept in dark at 29 °C for 14 hours. The solution was diluted to 65 folds with phosphate buffer, pH 7.4. 50 µl of the extract (125 – 2000 µg/ml) was taken and mixed with 3 ml of the above solution. After 20 minutes of incubation at room temperature, the absorbance was measured at 734 nm and percentage inhibition was calculated using equation 1<sup>21, 22</sup>.

### *Reducing power*

The reducing potential were determined using different concentrations of the extracts varying from 125 to 2000 µg/ml. 2.5 ml of each concentration (10 mg/ml) was mixed with 2.5 ml 1 % Potassium ferricyanide and 2.5 ml 0.2 M phosphate buffer (pH 6.6). The whole mixture was boiled for 20 minutes at 50 °C. After incubation, 2.5 ml of 10 % Tri chloroacetic acid (TCA) was added and centrifuged at 3000 rpm for 10 minutes. The upper layer was diluted with equal volumes of distilled water and 1 ml of 0.1 % ferric chloride. Absorbance measured at 700 nm is directly proportional to reducing ability of the extracts. Ascorbic acid was used as the standard for comparing the activity<sup>23-25</sup>.

## In vivo hepatoprotective activity analyses

### *Acute toxicity study*

Wistar albino female rats (120-150 g weight) were selected for the evaluation of hepatoprotective activity. All the experiments involving the use of animals were conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All the animals were maintained under controlled environmental conditions and had free access to sterile water and food. The protocol was approved by Institutional Animal Ethical Committee (IAEC) with CPSEA Reg. No. 1195 /Re /S /08 / CPCSEA. Organizations for Economic Co-operation and Development (OECD) guidelines No.425 was referred for acute toxicity studies and fix the dosage to conduct experiments. The plant extracts were orally administered with a gradual increasing dosage upto 2000

mg/kg body weight. Any physiological or toxicological changes were keenly observed for 14 days<sup>26</sup>.

#### *Experimental protocol*

Animals were randomly selected and assigned in 5 groups with each containing 6 animals. Group I- Served as negative control and received the vehicle (CMC 5 ml/Kg b.w) by gavage Group II to Group V - Administered CCl<sub>4</sub> 2ml/kg b.w (1: 1 dilution with liquid paraffin) s.c Group III- Standard group- Animals received silymarin in the dose of 50 mg/kg b.w Group IV- SVEE1- Received 200 mg/kg b.w extract by oral route Group IV- SVEE1- Received 400 mg/kg b.w extract by oral route

The extracts and standards administered orally once in a day for 14 days and CCl<sub>4</sub> was administered s.c at the lower abdomen to groups II to V after every 72 h<sup>27,28</sup>.

#### *Assessment of hepatoprotective activity*

The animals of all groups were sacrificed by cervical decapitation on 15<sup>th</sup> day. The blood samples were collected from each group of animals by intra cardiac puncture into sterilized and dried centrifugal tubes. Serum was separated and analyzed for biochemical investigation including SGOT (Serum Glutamate Oxaloacetate Transaminase) or AST (Aspartate Amino Transferase), SGPT (Serum Glutamate Pyruvate transaminase) or ALT (Alanine Amino Transferase), ALP (Alkaline Phosphatase), total protein, total bilirubin and direct bilirubin<sup>29,30</sup>.

#### *Histopathology*

The liver samples were excised from each group of animals after blood withdrawal and was washed with normal saline. Liver fragments were preserved in 10% formalin until use. 0.5 µm thickness sections were prepared by using microtome and were stained with hematoxylin-eosin dye. The sections were observed and photographs were taken under a light microscope for the assessment of histopathological studies<sup>29,30</sup>.

#### *Statistical analysis*

All the antioxidant studies were carried out in triplicates and the values were expressed in mean ± S.D.

Biochemical estimation results were recorded in mean ± SEM (n=6) and statistical analyses were done by one way ANOVA followed by Dunnett's test (SPSS software version 20). Differences were significant at the level of p < 0.01.

#### *GC-MS analysis*

GC-MS analysis was performed to identify the bioactive constituents of the ethyl acetate extract using Shimadzu GC-MS spectrometer GC-2010 equipped with DB 35-MS capillary standard non-polar poly (dimethylsiloxane) column (30 mm length × 0.25 mm in diameter × 0.25 µm in thickness of film). Pure helium was used as the carrier gas (flow rate of 1 ml/min). The injector operating temperature was adjusted to 280°C. The oven temperature was programmed to raise from 70°C to 280°C, at a rate of 10°C per/min.

#### *Identification of chemical constituents*

The chemical ingredients derived from the ethyl acetate extract of *S.violaceum* were analyzed. Compounds were identified based upon the GC retention time. The interpretation of GC chromatogram was carried out with NIST (National Institute of Standard and technology) library.

## RESULTS

### Qualitative analysis of phytochemicals

Qualitative analysis of phytochemicals was carried out in the extracts of the areal parts of *S.violaceum* and the results are given in table 1. The results reveal the presence of carbohydrates and proteins in the alcoholic and water extracts. The ethyl acetate, alcoholic and water extracts contain tannins, phenolics, flavonoids and saponins. While alkaloids are enriched only in the chloroform extract. While the petroleum ether and chloroform extracts were found to contain majorly the steroids.

### Quantitative analysis of phytochemicals

The total phenolic (TP) content was estimated in the extracts by folin-ciocaleau method and the results are given in table 2. The amount of phenolics was expressed in milligram Gallic acid equivalent per gram dry weight (mg GAE/g) of the extract and it was found to vary from  $85.07 \pm 1.106$  to  $215.3 \pm 0.819$  mg GAE/g. The highest phenolic content was found to be in the ethyl acetate extract.

Total flavonoid content (TFC) by aluminium chloride precipitation method was expressed as quercetin equivalent per gram dry weight (mg QE/g) of the extract. The higher concentration of flavonoid content was observed in chloroform and ethyl acetate extracts (Table 2).

### Evaluation of invitro antioxidant potential

#### *DPPH method*

The free radical scavenging ability of the extracts was evaluated by DPPH method with ascorbic acid as the standard antioxidant. The results are given in table 3 and depicted in figure 1. Ethyl acetate extract showed significant antioxidant activity with  $IC_{50}$  value of  $93.84 \pm 1.03$   $\mu\text{g/ml}$  which was comparable with the  $IC_{50}$  of standard ( $48.74 \pm 0.21$   $\mu\text{g/ml}$ ).

#### *Nitric oxide radical scavenging*

The free radical scavenging ability of the extracts towards nitrite was evaluated using Griess reagent and the percentage inhibition was calculated for each extract. There was a linear increase in the inhibitory activity towards increase in concentration of the extract. The ethyl acetate extract was found to exhibit significant nitric oxide scavenging potential with  $IC_{50} = 667.78 \pm 12.62$   $\mu\text{g/ml}$ , when compared to other extracts. The results are given in table 4 and figure 2.

#### *ABTS method*

This method measures the ability of the extracts to scavenge the ABTS radicals generated by the reaction between ABTS reagent and potassium per sulfate. In the present study, lowest  $IC_{50}$  value with significant antioxidant activity was observed for alcoholic extract ( $2671.28 \pm 12.969$   $\mu\text{g/ml}$ ) followed by ethyl acetate extract ( $2878.97 \pm 6.934$   $\mu\text{g/ml}$ ). All the extracts show significantly higher  $IC_{50}$  value (table 5 and figure 3) as compared to the standard, ascorbic acid.

#### *Reducing power method*

The reducing power of the standard, quercetin was reported to be in the range of  $1.05 \pm 0.001$  to  $2.44 \pm 0.0005$   $\mu\text{g/ml}$ . The results given in figure 4 reveal that all the extracts of *S.violaceum* show a dose dependent reducing power and the results are significantly comparable to the

standard. Among all the extracts, the relative reducing power was significantly high in the alcoholic extract and is in the range of  $0.4246 \pm 0.0016 \mu\text{g/ml}$  to  $1.5406 \pm 0.0012 \mu\text{g/ml}$ .

#### Acute toxicity study

The acute toxicity was determined by administering 2000 mg/kg body weight of the ethyl acetate extract from the aerial parts of extract *S.violaceum* in animal models. No signs of toxicity or mortality were observed for a period of 14 days. Also no behavioral changes were observed during this period. Therefore for further hepatoprotective evaluations only 1/10<sup>th</sup> and 1/20<sup>th</sup> doses of the extracts were used.

#### Hepatoprotective activity

Released biochemical parameters such as SGOT, SGPT, ALP, direct & total bilirubin and total protein content were measured in the serum of all groups of animals. The results are presented in table 6 & figure 5. In vehicle control group, the levels of SGOT ( $59.33 \pm 2.55$  IU/L), SGPT ( $52.83 \pm 2.33$  IU/L), ALP ( $101.5 \pm 3.23$  IU/L), total bilirubin ( $0.703 \pm 0.02$  mg/dl), direct bilirubin ( $0.163 \pm 0.006$  mg/dl), and total protein ( $8.47 \pm 0.02$  g/dl) was found to be at normal levels. The CCl<sub>4</sub> treated group showed significantly higher amounts of SGOT ( $145.83 \pm 4.45$  IU/L), SGPT ( $112.17 \pm 3.05$  IU/L), ALP ( $324.17 \pm 3.55$  IU/L), total bilirubin ( $1.42 \pm 0.05$  mg/dl), direct bilirubin ( $0.34 \pm 0.02$  mg/dl) and a significantly decreased production of total protein ( $6.28 \pm 0.03$  g/dl). All the liver enzymes contents were subsequently decreased by the administration of standard (silymarin) and *S.violaceum* ethyl acetate extract, except the total protein content.

#### Histopathology

The histopathological images (figure 6A) of normal control groups indicated the presence of normal hepatic cells with well-organized central vein and clear arrangement of hepatic cells. The CCl<sub>4</sub> toxicated groups (figure 6B) showed cell necrosis, loss of cell boundaries, degradation of central vein, ballooning degeneration and vacuoles of fatty degeneration. The liver sections of extract treated (figure 6C, 6D) showed liver with less necrosis, reduced crowding of central vein, and the results were significantly comparable with the normal group. Liver section of standard (silymarin) group showed a clear central vein with less cellular necrosis and these changes show similarity to the architecture of normal control group animals.

#### Characterization of compounds

GC-MS analysis was carried out for the identification of compounds which are present in the ethyl acetate extract, and exhibiting significant antioxidant activity. Table 7 presents the list of 20 compounds identified by the NIST library search and figure 7 depicts their chromatogram.

## DISCUSSION

The use of plants as a drug source is, as old as human civilization. The plants constitute a wide collection of chemical compounds with invincible pharmacological activity. But for their complete exploitation intensive efforts need to be taken. Plants are more familiar to traditional systems of medicines like ayurveda, unani, siddha etc. *Solanum violaceum* is one of the most important ingredients of several ayurvedic formulations. Most of the *Solanum* species are reported to possess high antioxidant and pharmacological activities. In this work, we have made an attempt to evaluate the antioxidant and hepatoprotective evaluation from the aerial parts of *S.violaceum*.

In the preliminary steps, the extracts were evaluated for the qualitative phytochemical analysis. This study revealed the presence of phyto constituents like alkaloids, glycosides, flavonoids, phenolics, tannins, steroids, saponin glycosides, etc. Recent studies claimed that the plants rich in polyphenolics and flavonoids are very essential for relieving oxidative stress. Further it has been observed and reported that such compounds could potentially lead to various toxicological changes like hepatic complications<sup>31</sup>.

Based upon these perceptions the plants were evaluated for quantification of flavonoids and total phenolic contents. Among the investigated extracts, the ethyl acetate extract showed the highest quantity of the above constituents and hence were evaluated for the antioxidant activity.

Antioxidants have remedial ability to scavenge free radicals generated by the body during metabolic processes and by eliminating oxidative stress<sup>19</sup>.

Antioxidants are preferred to investigate the CCl<sub>4</sub> induced liver toxicity and its mechanism in preventing chain reaction and lipid peroxidation. This concept provides an idea for the evaluation of antioxidant activity study in the extracts. DPPH, ABTS, NO radical scavenging and reducing power methods were carried out for the antioxidant studies. Among the extracts, ethyl acetate extracts showed significant antioxidant activity and hence the same is selected for further hepatoprotective evaluation with CCl<sub>4</sub> intoxicated models<sup>32</sup>.

CCl<sub>4</sub> intoxicated models are the most widely used hepatic models for the hepatoprotective studies. CCl<sub>4</sub> gets metabolized by the liver and produces trichloromethyl radicals. This binds with lipids and proteins and lead to the peroxidation of polyunsaturated fatty acid constituents of the endoplasmic reticulum membrane and this lipid peroxidation leads to the damage of hepatic cells<sup>33, 34</sup>.

Kupffer cell activation also plays an important role in the development of secondary damage of the hepatic cells. The liver cells, such as hepatocyte, endothelial cells and stellate cells are getting activated by the release of proinflammatory mediators that activates the kupffer cell. This, in turn, activates inflammatory mediators, especially TNF- $\alpha$ <sup>35</sup>. The above facts support the significant hepatoprotective activity of the ethyl acetate extract of the aerial parts of *Solanum violaceum*.

GC-MS studies of the ethyl acetate extracts revealed the presence of 20 compounds with four prominent constituents. The important constituents include viridiflorol, Palmitic acid, n-pentacosanal, Citroflex A. The antioxidant and hepatoprotective potential of the above compounds have been reported in the literature<sup>36-38</sup>.

Our results support the hepatoprotective potential of *Solanum violaceum* by its free radical scavenging potential. The assessment of the key parameters of serum enzymes concluded that the ethyl acetate extract has a major role in liver protection through the activation of antioxidant defense system. The effects were comparable with the standard hepatoprotective drug, silymarin. Further evaluation has to be carried out for selecting *Solanum violaceum* as a future remedy for liver protection against hazardous chemicals and other xenobiotics.

#### **Author contribution**

The authors have equally contributed to the work reported in this manuscript.

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## Abbreviations

CCl <sub>4</sub>	Carbon tetrachloride
SVEE	<i>Solanum violaceum</i> ethyl acetate extract
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
DPPH	2, 2- diphenyl-1-picryl hydrazyl
NO	Nitric oxide
ABTS	2 and 2-Azino-bis [3-ethyl benzothiazoline-6-sulfonic acid]
ROS	Reactive Oxygen Species
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent
SGOT	Serum Glutamate Oxaloacetate Transaminase

SGPT Serum Glutamate Pyruvate Transaminase  
ALP Alkaline Phosphatase  
CMC Carboxy Methyl Cellulose  
b.w Body Weight  
s.c Subcutaneous

Table 1: Qualitative phytochemical analysis from the aerial parts of *S.violaceum*

Constituents	Extracts				
	Petroleum Ether	Chloroform	Ethyl acetate	Alcohol	Water
Carbohydrates	-	-	-	+	+++
Proteins	-	-	-	+	++
Tannins and phenolics	-	-	+++	++	+
Flavonoids	-	-	+++	++	+
Alkaloids	-	++	-	-	-
Volatile oils	-	-	-	-	-
Steroids	+++	++	-	-	-
saponins	-	-	+	+	+

Table 2: Quantitative estimation of total phenolic and flavonoids in *S.violaceum* extracts

Sl.No	Sample	Total Phenolic content (mg GAE/g)	Flavonoid content (mg QE/g)
1.	Petroleum ether	85.07 ± 1.106	65.23 ± 0.666
2.	Chloroform	162.57 ± 1.234	133.83 ± 1.193
3.	Ethyl acetate	215.3 ± 0.819	125.27 ± 0.709
4.	Alcohol	175.7 ± 1.769	71.7 ± 0.802
5.	Water	151.37 ± 1.07	70.87 ± 0.651

All values are expressed as mean ± S.D of three replicates

Table 3: Free radical scavenging of *S.violaceum* extracts by DPPH method

Sl.No.	Sample	IC <sub>50</sub> (mean ± S.D) (µg/ml)
1.	Standard (Ascorbic acid)	48.74 ± 0.21
2.	Petroleum ether	444.18 ± 3.46
3.	Chloroform	273.85 ± 1.56
4.	Ethyl acetate	93.84 ± 1.03
5.	Alcohol	360.34 ± 5.13
6.	Water	327.19 ± 1.93

Table 4: Nitric oxide radical scavenging activity of *S.violaceum*

Sl.No.	Sample	IC <sub>50</sub> (mean ± S.D) (µg/ml)
1.	Standard (Gallic acid)	147.11 ± 10.20
2.	Petroleum ether	3605.33 ± 7.57
3.	Chloroform	1792.5 ± 19.53
4.	Ethyl acetate	667.78 ± 12.62
5.	Alcohol	2401.67 ± 4.41
6.	Water	1679.99 ± 15.28

Table 5: Antioxidant activity evaluation from the aerial parts of *S.violaceum* by ABTS method

Sl.No.	Sample	IC <sub>50</sub> (Mean ± S.D) (µg/ml)
1.	Standard (Ascorbic acid)	941.09 ± 8.312
2.	Petroleum ether	5068.713 ± 14.68
3.	Chloroform	2943.33 ± 4.506
4.	Ethyl acetate	2878.97 ± 6.934
5.	Alcohol	2671.28 ± 12.969
6.	Water	6157.14 ± 5.580

Table 6: Measurement of SGOT, SGPT, ALP, Bilirubin and total protein contents of the extracts

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Bilirubin (mg/dl)		TOTAL PROTEIN (g/dl)
				TOTAL	DIRECT	
Vehicle control	59.33 ± 2.55	52.83 ± 2.33	101.5 ± 3.23	0.703 ± 0.02	0.163 ± 0.006	8.47 ± 0.02

<b>Toxic Control</b>	145.83±4.45	112.17±3.05	324.17±3.55	1.42±0.05	0.34±0.02	6.28±0.03
<b>Standard(Silymarin)</b>	66.33±2.19**	56.67±2.20**	109.83±3.21**	0.751±0.01**	0.17±0.01**	8.36±0.02**
<b>SVEE (200mg/kg)</b>	81±1.77**	69.5±1.84**	131±1.32**	0.85±0.01**	0.147±0.01**	8.09±0.01**
<b>SVEE(400mg/kg)</b>	67.67±1.99**	61.83±1.14**	126±1.32**	0.72±0.02**	0.13±0.01**	8.16±0.01**

Values are expressed as Mean±SEM of 6 rats in each group. SVEE: *S.violaceum* aerial part ethyl acetate extract, SGOT: Serum Glutamate Oxaloacetate Transferase, SGPT: Serum Glutamate Pyruvate Transferase, ALP: Alkaline Phosphatase.

\*\*P< 0.05 as compared with Toxic control group.

Table 7: List of compounds identified by GC-MS analysis of the ethyl acetate extract of *S.violaceum* aerial parts

Retention time (Minutes)	IUPAC name	Common name	Molecular formula	PK area (%)
11.991	(1aR,4S,4aR,7R,7aS,7bS)-1,1,4,7-tetra methyldecahydro-1H-cyclopropa[e]azulen-4-ol	Globulol	C <sub>15</sub> H <sub>26</sub> O	3.85
13.775	Ethyl p-methoxycinnamate	Ethyl 4-Methoxycinnamate	C <sub>12</sub> H <sub>14</sub> O <sub>3</sub>	7.25
15.349	Thunbergol	Isocembrol	C <sub>20</sub> H <sub>34</sub> O	3.03
15.838	Cyclohexanol, 5-methyl-2-(1-methyl-1-phenylethyl)-	8-phenylmenthol	C <sub>16</sub> H <sub>24</sub> O	6.59
15.933	n-Hexadecanoic acid	Palmitic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	11.05
16.300	Hexadecanoic acid, ethyl ester	Ethyl palmitate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	3.53
16.569	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	viridiflorol	C <sub>15</sub> H <sub>26</sub> O	8.38
17.957	Octadecanoic acid	Stearic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	3.98
18.003	Oxacycloheptadec-8-en-2-one, (8Z)-	ambrettolide	C <sub>16</sub> H <sub>28</sub> O <sub>2</sub>	6.18
18.753	Tributylacetyl citrate	Citroflex A	C <sub>20</sub> H <sub>34</sub> O <sub>8</sub>	8.05
19.537	Docosanal	Docosyl aldehyde	C <sub>22</sub> H <sub>44</sub> O	2.81
20.044	2-Oxo-5-benzoyl-6-phenyl-4-(4-tolyl)-1,2,3,4-tetrahydropyrimidine	-	C <sub>24</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>	2.56
20.430	Henicosan	Henicosan	C <sub>21</sub> H <sub>42</sub> O	7.26
21.008	Octacosane, 2-methyl-	Isononacosane	C <sub>29</sub> H <sub>60</sub> O	2.19
21.297	Pentacosanal	n-pentacosanal	C <sub>25</sub> H <sub>50</sub> O	11.12
22.233	Hexadecanal	palmitaldehyde	C <sub>16</sub> H <sub>32</sub> O	2.32
22.895	Nonyloctacosyl ether	-	C <sub>37</sub> H <sub>76</sub> O	1.98
23.515	Acetic acid, chloro-, octadecyl ester	Octadecylchloroacetate	C <sub>20</sub> H <sub>39</sub> ClO <sub>2</sub>	2.77
28.329	Cholesta-4,6-dien-3-ol, (3.β.)-	4,6-cholestadienol	C <sub>27</sub> H <sub>44</sub> O	2.21
29.057	Cholesterol	Cholesteryl alcohol	C <sub>27</sub> H <sub>46</sub> O	2.90

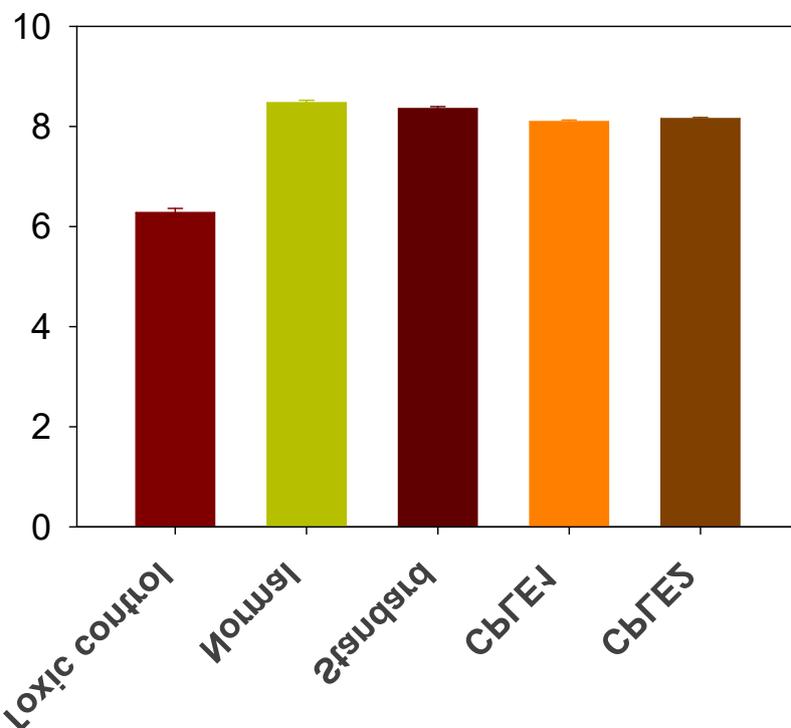


Figure 1: The bar graph representing the antioxidant activity of *S.violaceum* by DPPH method.

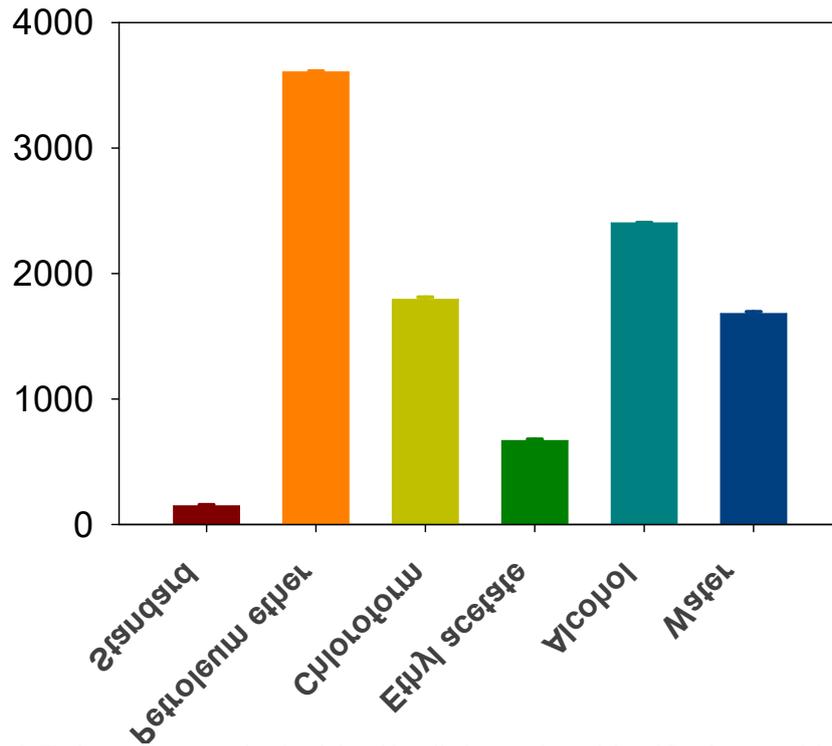


Figure 2: The bar graph representing the nitric oxide radical preventive activity of *S. violaceum* aerial parts.

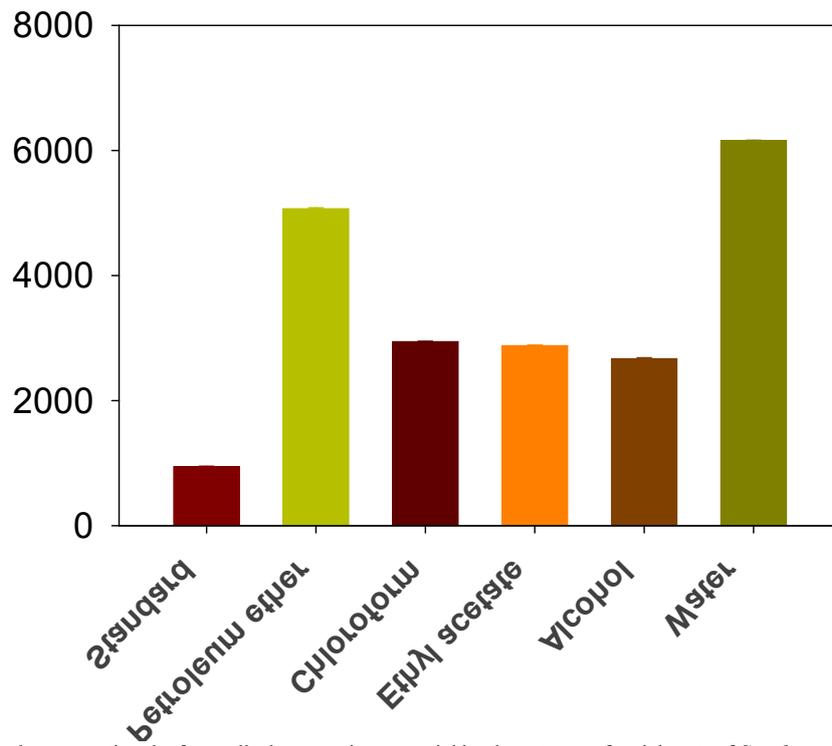


Figure 3: The bar graph representing the free radical scavenging potential by the extracts of aerial parts of *S. violaceum* by ABTS method.

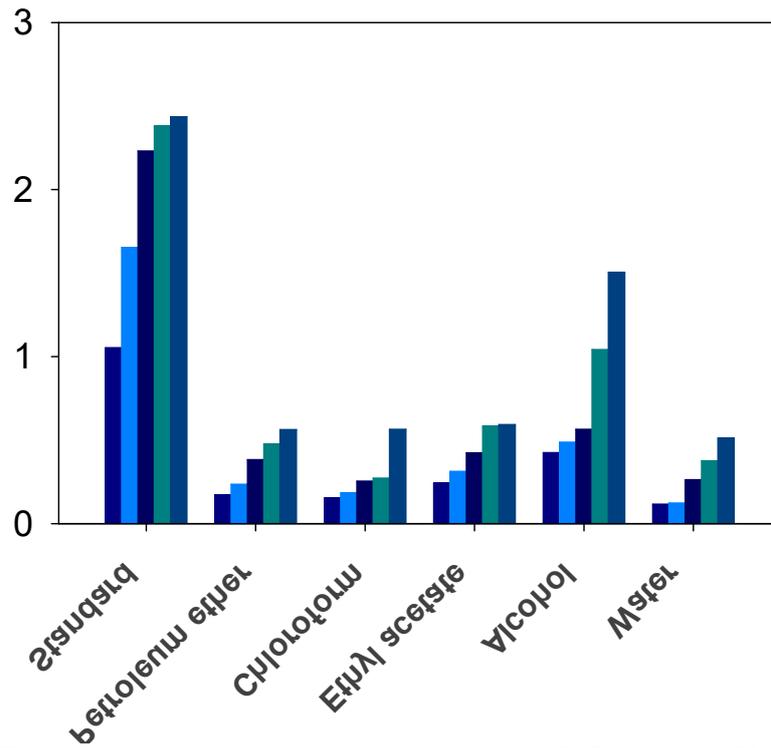


Figure 4: The bar graph representing the dose dependent reducing ability of different extracts of *S.violaceum*.

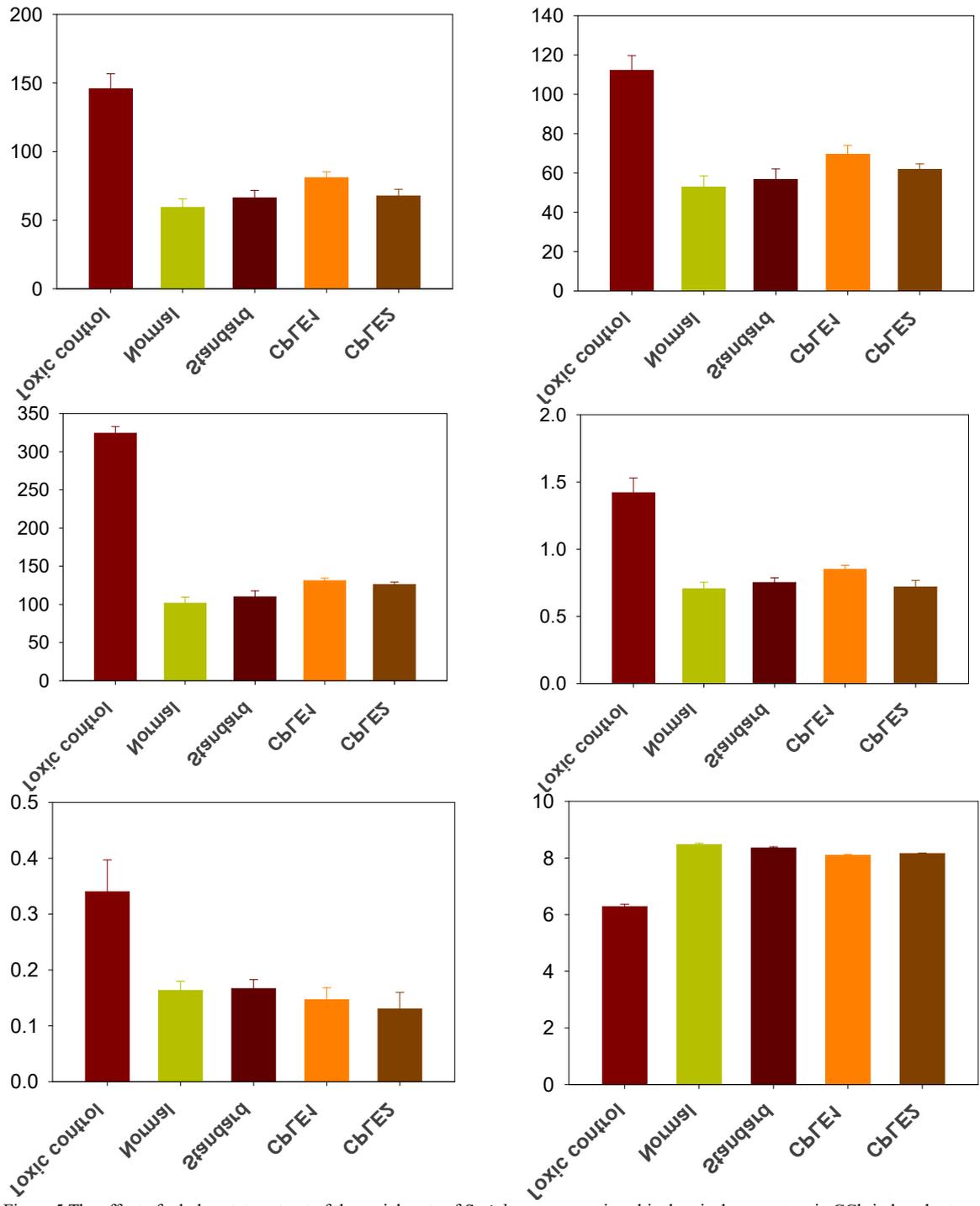


Figure 5. The effect of ethyl acetate extract of the aerial parts of *S. violaceum* on various biochemical parameters in CCl<sub>4</sub> induced rat models. Values are mean (IU/L) ± S.E.M (n=6).

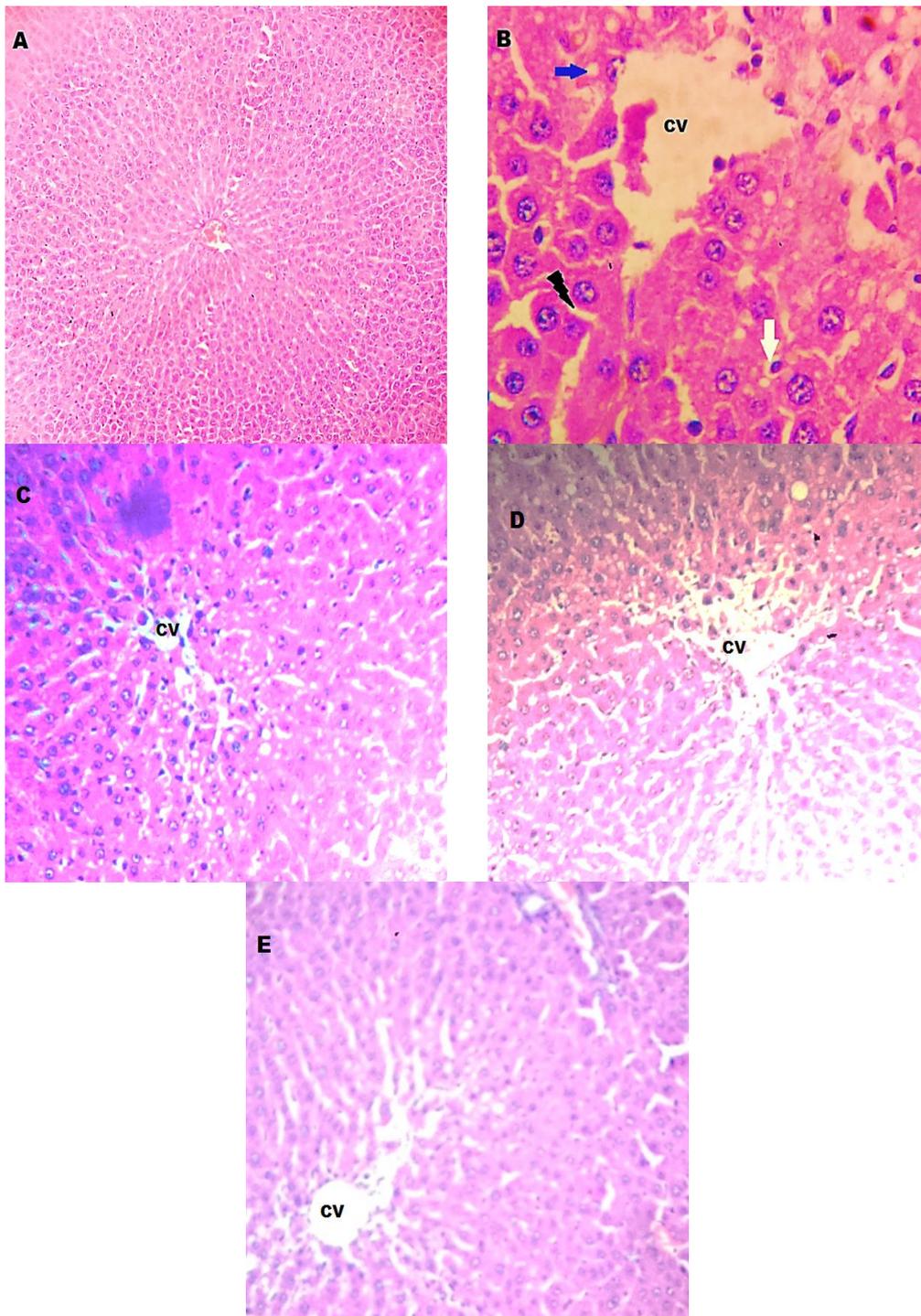


Figure 6: Photomicrographs of stained liver sections. 6A. Vehicle control group displaying normal histological liver structure. 6B. CCl<sub>4</sub> toxicated liver section showing cell necrosis (blue arrow), loss of cell boundaries, central vein degradation, ballooning degeneration (white arrow), and sinusoidal degeneration (black arrow). 6C & 6D. The liver sections treated with extract displaying less necrosis and reduced crowding of central vein. 6E. Liver section of standard showing a clear central vein with less cellular necrosis.

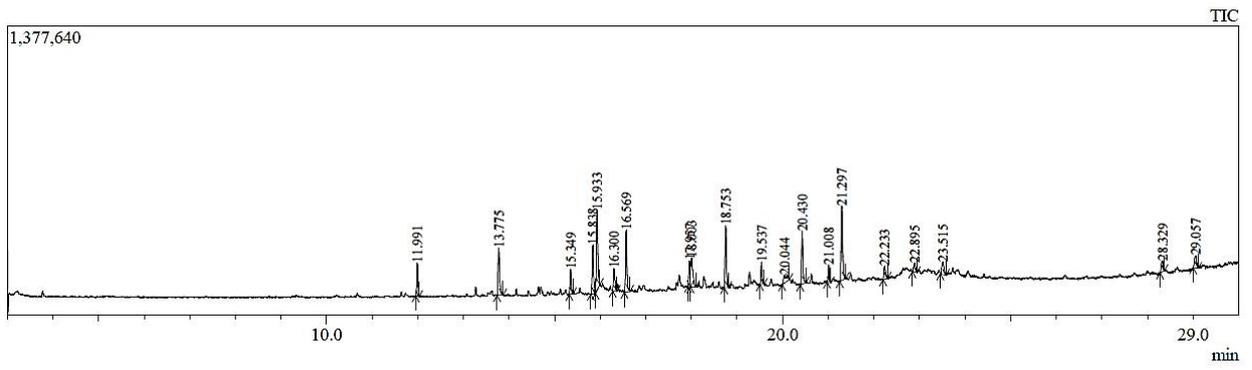


Figure 7: GC-MS chromatogram of the ethyl acetate extract from the aerial parts of *S. violaceum*.