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*Full Length Research Paper*

# Extraction and identification of the hepatoprotective bio-active components of the root of *Actinidia deliciosa*

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**The root of *Actinidia deliciosa* has been used as traditional drugs in China for a long time. This study therefore aimed at investigating the hepatoprotective bio-active components from the root of *A. deliciosa* comprehensively and accurately, thus promoting the exploitation and utilization of the whole resource of *A. deliciosa*. For the purpose of this study, the roots of *A. deliciosa* were fractionated into various extract fractions with differential polarity solvent. The hepatoprotective activities of various solvent extracts were assessed by examining the effect on carbon tetrachloride ($CCl_4$)-induced hepatotoxicity in rats. The highest hepatoprotective activities fraction was further isolated with column chromatography. The structures of the activities monomers were identified with modern spectrum technology such as infrared (IR), ultraviolet (UV), electron impact mass spectrometry (EI-MS), proton nuclear magnetic resonance ($^1$H-NMR), carbon nuclear magnetic resonance ($^{13}$C-NMR) and distortionless enhancement by polarization transfer-nuclear magnetic resonance (DEPT-NMR). Of the various extract fractions, the ethyl acetate extract (Fr3) exhibited the highest hepatoprotective activities ($p$<0.05). When the Fr3 was separated into five fractions by silica gel chromatography, among the five fractions fraction, Fr9 showed the highest yield and the highest hepatoprotective activities. When using Fr9 at a dose of 200 mg/kg to pre- and post-treat the $CCl_4$-induced rat, the activities of alanine transaminase (ALT) decreased by 90.10 and 88.60%, aspartate transaminase (AST) decreased by 80.69 and 79.92% in rat serum, the lipid peroxidation (malondialdehyde (MDA)) decreased by 42.11 and 45.53%, while glutathione (GSH) increased by 114.12 and 147.62% in the rats liver homogenate, respectively as compared with that of the $CCl_4$ control rats. The fraction Fr9 was further separated into five fractions using silica gel chromatography, which were investigated for the main chemical constituents by column chromatography techniques, physico-chemical constants and spectroscopic analysis. Its main chemical constituents were three triterpenoids named 3β-hydroxy-urs-12-en-28-oic acid (ursolic acid), 2α,3α,23-trihydroxy-urs-12-en-28-oic acid and 2α,3β,19,23-tetrahydroxy-urs-12-en-28-oic acid. The findings indicate that the high hepatoprotective activity of the fraction of *A. deliciosa* root is due to its enriched triterpenoid.**

**Key words:** *Actinidia deliciosa* root, carbon tetrachloride ($CCl_4$), hepatoprotective, Chemistry.

## INTRODUCTION

Liver intoxication has increased as a result of exposure to high levels of environmental toxins, since the liver has an important role in detoxification and most of the liver damage are induced by lipid peroxidation and other oxidative damages caused by the hepatotoxic chemicals (Appiah et al., 2009; Muhtaseb et al., 2008; Subramoniam and Pushpangadan, 1999). Carbon tetrachloride ($CCl_4$) is a well know hepatotoxin that is widely used to study the induction of toxic liver injury in a range of laboratory animals (Appiah et al., 2009). Antioxidation plays an

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important role in protecting against carbon tetrachloride-induced liver injury (Ardanaz and Pagano, 2006).

In recent years, Chinese herbal medicine has attracted many attentions from researchers in various areas. A number of medicinal preparations in Chinese herb have been recommended for the treatment of liver disorders (Chatterjee, 2000). *Actinidia deliciosa* is subfamily of the genus *Actinidia*, and is widely distributed in the Asian continent. The fruit of *A. deliciosa* has been acclaimed for its native and medicinal values. The root of *A. deliciosa* has been used as traditional drugs in China for a long time; it was reported as a folk remedy for adult diseases, as a potent anti-hepatotoxic, and against pyorrhea and gingival inflammation (Jiangsu New Medical College, 1997). Furthermore, the ethanol extracts of *A. deliciosa* root had been proven to possess anticancer properties *in vitro* (Zhong et al., 2004) and *in vivo* (Li, 2001). The active compounds from *A. deliciosa* have been separated and identified; 5 compounds were isolated and identified as: 2α,3β,19,23-tetrahydroxylolean -12-en-28-oic acid (I), 2α,3α,23-trihydroxy-urs-12-en-28-oic acid(II), 2α,3β,19,23 -tetrahydroxyl-urs-12en-oic acid(III), undecanoic acid(IV), beta-sitosterol (V) (Lai and Xu, 2007). The current authors have also reported that the ethanol extracts from roots of *A. deliciosa* have anti-hepatotoxic effects (Bai et al., 2007), and could have effect on liver pathological changes (Bai and Qiu, 2006). However, the hepato-protective main chemical constituents of *A. deliciosa* root have not been previously investigated in a systematic way.

Based on our previous studies (Bai et al., 2007; Bai and Qiu, 2006), we envisaged a study of the hepato-protective potentials and chemistry of the plant, particularly its root parts, so as to provide a scientific basis to its usefulness in ameliorating the liver dys-function. Therefore, in the present study, we utilized various exactions obtained from *A. deliciosa* root for an evaluation of its hepatoprotective potentials, employing a sequence of tests in various experimental models of carbon tetrachloride ($CCl_4$)- induced hepatotoxicity. A bioassay based fractionation of 60% ethanol extract of *A. deliciosa* root led us to the identification of active fraction here after called as 'TF'. The bioactive fraction has been standardized on the basis of their physical characteristics and spectral data to obtain three major triterpenoid namely 3β-hydroxy-urs-12-en-28-oic acid (I); 2α,3α,23-trihydroxy-urs-12-en-28-oic acid (II) and 2α,3β,19,23-tetrahydroxy-urs-12-en-28-oic acid (III) (Figure 1). This paper reports the therapeutic potential of TF as a hepatoprotective agent and its main chemical constituents.

## MATERIALS AND METHODS

### Chemicals

Assay kits for measuring serum alanine transaminase (ALT), aspartate transaminase (AST) activities, hepatic lipid peroxidation (malondialdehyde (MDA)) and glutathione (GSH) were purchased from Nanjing Biomedical. Ltd. (Nanjing, China). The standard ursolic acid was obtained from Sigma Chemical Co. (St Louis, MO, USA.). All other solvents and chemicals used were of analytical grade and purchased from Shanghai Chemicals Co. (Shanghai, China)

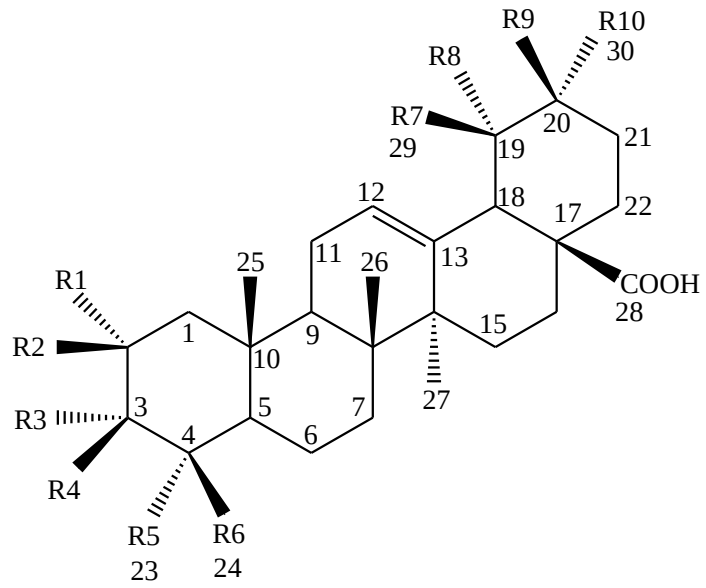
### Collection of plant material

The roots of *A. deliciosa* were collected from the plants grown in Hainan University Horticultural Garden (Hainan Province, China) in November, 2009, and identified by Prof. Liu Zhonghua of the Department of Botany, Hainan University. Root materials were separated, washed, cut in to small part, air-dried (moisture 10% in weight), ground by a miller (A11 basic, ZKA®-WERKE, Germany), screened by sieve, and the particles of (0.5-1.5) $\times 10^{-2}$ mm diameter were selected. A voucher specimen had been deposited in the key Laboratory of Food Science and Safety, Hainan University, Hainan China, Vide accession No. 2009069.

### Preparation of extracts and identification of active fraction

The dried and powdered *A. deliciosa* root (10 kg) was extracted with 60% ethanol (v/v) (60 L ×3) under 45°C for 8 h each time. Next, the combined extract was cooled to room temperature (25°C), filtered through muslin and then the filtrate was concentrated under reduced pressure (45°C, 0.1 MPa, 3 h) and freeze-dried (24 h) to produce a 60% ethanol crude extract (Fr1) (2.06 kg). The yield on the dry root corresponded to 20.60%. The extract (Fr1) (1000 g) so obtained was suspended in water (3000 ml) and extracted successively with n-hexane, ethyl acetate and n-butanol (3 × 5 L each) to obtain four fractions: a n-hexane extract (Fr2) (89.2 g, yield, 1.84%), an ethyl acetate extract (Fr3) (348.6 g, yield, 7.18%), *n*-butanol extract (Fr4) (229.5 g, yield, 4.73%) and aqueous portion (Fr5) (257.3 g, yield, 5.30%) after removal of the solvent in vacuum. All the fractions were subjected to screening of hepatoprotective activity (preventive and curative study) against $CCl_4$ induced hepatic injury in rats on limited test models, which led to establishing that Fr3 and Fr4 possessed significant hepatoprotective activity, while the Fr3 was more potent over the Fr4.

Keeping in view the percent yield and bio-activity, the Fr3 was further taken up for detailed hepatoprotective activity. The active fraction Fr3 (175 g) was also subjected to column chromatography on Diaion HP-20 with a successive elution system of water: ethanol (100:0 to 5:95) to obtain five fractions (Fr6 - Fr10): Fr6 (14.6 g, yield, 0.6%), Fr7 (21.7 g, yield, 0.89%), Fr8 (30.5g, yield, 1.25%), Fr9 (TF, 50:50 to 30:70 elution part) (52.6 g, yield, 2.16%) and Fr10 (26.2 g, yield, 1.07%). All these fractions were biologically evaluated against $CCl_4$-induced hepatic injury in rats (prophylactic study) for ALT, AST, hepatic lipid peroxidation and GSH to identify the active fraction eliciting maximum hepato-protection, which was taken up for further studies. On detailed chemical analysis, the active fraction Fr9 (TF) was found to be a mixture of triterpenoid, resolving into three major spots on thin layer chromatography (TLC) (chloroform: methanol, 70:30, one spots $R_f$ values were equivalent to ursolic acid) and some minor spots. Fr9 (TF) (20 g) was subjected to column chromatography on Sephadex-LH-20 for the separation and identification of individual components. On elution with water: ethanol (90: 10 to10: 90), the three major fractions FrI, FrII and FrIII could be separated. Fraction FrI, FrII and FrIII were purified by silica gel H and RP-18 gel column chromatography to give compound I (450 mg), compound II (210 mg) and compound III (150 mg). These compounds were identified on the basis of their physical characteristics and spectral data.




| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 |
|---|---|---|---|---|---|---|---|---|---|---|
| I | H | H | H | OH | Me | Me | Me | H | H | Me |
| II | OH | H | OH | H | $CH_2OH$ | Me | Me | H | H | Me |
| III | OH | H | H | OH | $CH_2OH$ | Me | Me | OH | H | Me |

**Figure 1.** Structure of compounds I, II and III.

**Animal treatment**

Charles Foster rats (150 - 180 g) of either sex, procured from Hainan Medical University (SCXK(Su)2009-0004, Hainan China) were used for the study. The animals were housed in large polypropylene cages and allowed free access to Purina Rodent Chow and tap water, maintained in a controlled environment at 20 ± 2°C and 50 ± 5% relatively humidity with a 12-h dark/light cycle, and acclimatized for at least one week before use. The grouping of the animals and the treatment regimens both for pre- and post-treatment of extracts fractions and silymarin against $CCl_4$ hepatotoxins is described in Table 1. A different set of animals was used for each experimental model. Freshly prepared suspension of extracts (2% w/v) and TF (1%, w/v) in normal saline were used in all the experiments against hepatotoxins; the control animals were given the vehicle only (Table 1).

**Induction of hepatic damage**

Liver injury was produced by administration of $CCl_4$ mixed with olive oil (1:1). A single dose of $CCl_4$ (1 ml/kg) was given to rat in both in preventive and curative studies by gastric intubation. The control animals received an equal volume of olive oil (Vogel, 1977) (Table 1).

**Preparation of serum and liver homogenate**

Blood was collected from the orbital sinus of all the animals and the serum was separated for various biochemical estimations. After the collection of blood, animals were sacrificed by decapitation and the liver was removed free of adhering tissues, perfused with cold saline, cut into small pieces blotted and weighed, and then minced and homogenized in glass homogenizer at 1100 rpm for 2 min in cold 10 mM Tris-HCl buffer (pH 7.4) so as to obtain 10% WH (Schenkman and Cinti, 1978) for the estimation of GSH and lipid peroxidation.

**Assay of hepatoprotectivity**

Serum was used for assaying ALT and AST (Reitman and Frankel, 1957), and the liver whole homogenate for lipid peroxidation (Wills, 1969; Fairhurst et al., 1982) and GSH (Ellman, 1959; Sedlak and Lindsay, 1968).

**Statistical analysis**

The data were expressed as mean ± standard error of mean (S.E.M; n = 6). Results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison using SPSS software student's version. The *p* value of < 0.05 or less was taken as the criterion of significance.

**Identification of active fraction main compound**

Melting points were taken on the Japanese Yanaco micro melting point apparatus and uncorrected. Infrared (IR) spectra were recorded on the Hitachi 270~50 Infrared Spectrophotometer in KBr discs. Electron impact mass spectrometry (EI-MS) data were recorded on the HP 5989A, JMS-D300 and Variam MAT 212 instrument. Proton nuclear magnetic resonance ($^1$H NMR) and carbon nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded with BRUKER AM-400 and/or DRK-500 Spectrometer using tetramethylsilane (TMS) as internal standard.

**Table 1.** Grouping and treatment schedule of animals in various hepatoprotective models.

| Group | Treatment (n = 6) | Prophylactic and Curative Multi dose treatment with TF + |
|---|---|---|
| Group I | Control vehicle only | Vehicle, |
| Group II | Control vehicle + $CCl_4$ | Vehicle |
| Group III | Silymarin + $CCl_4$ | Reference 60 mg/kg, p.o. |
| Group IV | TF + $CCl_4$ | TF 25 mg/kg, p.o (77891* mg/kg) |
| Group V | TF + $CCl_4$ | TF 50 mg/kg, p.o. (155782*mg/kg) |
| Group VI | TF + $CCl_4$ | TF 100 mg/kg, p.o.(311565*mg/kg) |
| Group VII | TF + $CCl_4$ | TF 200mg/kg, p.o.(623130*mg/kg) |

TF indicate the bio-active fraction isolated from *A. deliciosa* root (suspension in normal saline). * The doses presented in the tables were normalized with *A. deliciosa* root raw materials. Vehicle: Normal saline; silymarin were used as positive standard to compare the results. Number of animals in each group is six (n = 6). Treatment was given orally by gastric intubation as per schedule given as follows: For prophylactic study, the test, reference drug and vehicle were given orally 48, 24 and 02 h, before and 06 h after hepatotoxin; blood and liver samples were collected 18 h after last treatment of test material for different estimations. For curative study, the test, reference drug and vehicle were given orally at 06, 24 and 48 h after hepatotoxin; blood and liver sample collected 02 h after the last treatment of test material for different estimations. Plant extracts: Water-ethanol, hexane, ethyl acetate, n-butanol, Marc and fractions of ethyl acetate (Fr6-10) were tested at the dose of 60 mg/kg, p. o. each. (* the doses presented in the table were normalized with *A. deliciosa* root raw materials).

**Table 2.** Effect of oral administration of different extracts from *A. deliciosa* root and silymarin on biochemical parameters against $CCl_4$-induced hepatic injury in rats (prophylactic and curative study).

| Treatment | Dose (mg/kg) | Prophylactic study (Serum parameters) | | Curative study (Serum parameters) | |
|---|---|---|---|---|---|
| | p.o. | ALT(U/L) | AST(U/L) | ALT(U/L) | AST(U/L) |
| Vehicle control | – | 80.96 ± 15.68 | 95.35 ± 8.49 | 89.72 ± 15.53 | 100.35 ± 12.45 |
| Vehicle+ $CCl_4$ | – | 586.23 ± 61.23$^{a}$ | 669.16 ± 33.63$^{a}$ | 1364.95 ± 73.26$^{a}$ | 717.01 ± 68.02$^{a}$ |
| Silymarin+ $CCl_4$ | 60.0 | 310.12 ± 19.82$^{ab}$ | 376.09 ± 29.90$^{ab}$ | 611.32 ± 32.63$^{ab}$ | 406.12 ± 31.46$^{ab}$ |
| Fr1+$CCl_4$ | 60.0(291*) | 389.43± 25.81$^{abcd}$ | 478.99 ± 41.24$^{acd}$ | 714.37 ± 19$^{abcd}$ | 475.68 ± 40.26$^{abcd}$ |
| Fr2 +$CCl_4$ | 60.0(15829*) | 412.68 ± 33.64$^{abcd}$ | 620.29 ± 64.83$^{acd}$ | 948.86 ± 42.23$^{abcd}$ | 574.21 ± 29.86$^{abcd}$ |
| Fr3+$CCl_4$ | 60.0(4056*) | 233.34 ± 19.79$^{abc}$ | 305.25 ± 23.76$^{abc}$ | 605.52 ± 45.15$^{ab}$ | 393.12 ± 24.62$^{ab}$ |
| Fr4□□+$CCl_4$ | 60.0(6158*) | 267.67 ± 24.84$^{abcd}$ | 315.70 ± 36.82$^{ab}$ | 522.98 ± 37.08$^{abcd}$ | 377.13 ± 39.21$^{ab}$ |
| Fr5 +$CCl_4$ | 60.0(5495*) | 303.32 ± 17.41$^{abd}$ | 360.29 ± 52.42$^{abd}$ | 614.33 ± 36.24$^{ab}$ | 349.36 ± 28.53$^{abcd}$ |

*The doses presented in the tables were normalized with *A. deliciosa* root raw materials. Values are expressed as mean ± S.E.M. of six animals in each group. Letters represent statistical significance: $^{a}$ $p < 0.01$, significantly different from the vehicle control. $^{b}$ $p < 0.01$, significantly different from the vehicle+ $CCl_4$; $^{c}$ $p < 0.01$, significantly different from the silymarin+ $CCl_4$; $^{d}$ $p < 0.05$, significantly different from the ethyl acetate extract + $CCl_4$. Dunnett's t test was used against the respective control.

Chromatographic separations were carried out on Diaion HP-20 (Nanjing, China), Sephadex-LH-20 (Shanghai, China), silica gel H and RP-18 gel column and TLC on silica gel $GF_{254}$ (Yantai, China).

## RESULTS AND DISCUSSION

### Effect of various extracts of *A. deliciosa* root on serum biochemical parameters against $CCl_4$-induced hepatic injury in rats (prophylactic and curative studies)

The results of the studies are summarized in Table 2. With treatment by $CCl_4$, the activities of ALT and AST in rat's serum increased by 624.00 and 601.80% (in prophylactic studies), and 1421.34 and 614.51% (in curative studies) compared to that of the normal control group, respectively. This result indicates that a single dose of $CCl_4$: olive oil (1:1, 1 ml/kg) causes hepatotoxicity in rats. The activities of ALT and AST were significantly ($p < 0.01$) increased in $CCl_4$ control compared to normal control. Moreover, the results with different extracts showed that all the extracts are effective against the acute $CCl_4$-induced hepatic damage in rats as evidenced from recovery of altered parameters. However, the Fr3 and Fr4 produced comparable protection that the effect is relatively higher than other extracts and reference drugs. Of five different extracts, Fr3 had higher hepatoprotective effect than Fr1, Fr2, Fr4 and Fr5 at the dose of 60 mg/kg ($p<0.05$) (Table 2). Fr3 has been proven to be more efficient on the decrease of ALT and AST. With pre-treatment of Fr3, the activities of ALT and AST from 586.23 ± 61.23 and 669.16 ± 33.63 U/L decreased to 233.34 ± 19.79 and 305.25 ± 23.76 U/L by 60.20 and 58.87%. Additionally, with post-treatment of Fr3, the activities of ALT and AST from 1364.95 ± 73.20 and 717.01 ± 68.02 U/L decreased to 605.52 ± 45.15 U/L and

**Table 3.** Effect of oral administration of the fractions from ethyl acetate extract (Fr3) from *A. deliciosa* root and silymarin on biochemical parameters against $CCl_4$-induced hepatic injury in rats (prophylactic study).

| Treatment | Dose (mg/kg) p.o. | Serum parameter | | Hepatic parameter | |
|---|---|---|---|---|---|
| | | ALT(U/L) | AST(U/L) | Lipid peroxidation (MDA: nmol/g liver) | Glutathione (µmole/g liver) |
| Vehicle control | – | 120.86 ± 11.20 | 124.68 ± 12.59 | 59.32 ± 2.15 | 6.13 ± 0.22 |
| Vehicle+ $CCl_4$ | – | 1120.68 ± 57.19$^{a}$ | 1042.69 ± 39.56$^{a}$ | 119.12 ± 3.59$^{a}$ | 2.27 ± 0.12$^{a}$ |
| Silymarin+ $CCl_4$ | 60.0 | 578.2 ± 41.50$^{ab}$ | 599.41 ± 34.99$^{ab}$ | 88.07 ± 3.64$^{ab}$ | 4.59 ± 0.38$^{ab}$ |
| Fr6+ $CCl_4$ | 60.0(676096*) | 860.45 ± 48.42$^{abcd}$ | 784.8 ± 56.53$^{abcd}$ | 99.51 ± 6.32$^{abcd}$ | 3.06 ± 0.15$^{abcd}$ |
| Fr7+ $CCl_4$ | 60.0(455795*) | 633.64 ± 48.48$^{abcd}$ | 562.91 ± 43.28$^{abd}$ | 88.98 ± 2.03$^{abd}$ | 3.60 ± 0.19$^{abcd}$ |
| Fr8+ $CCl_4$ | 60.0(324526*) | 598.22 ±40.19$^{abd}$ | 590.42 ± 47.36$^{abd}$ | 87.08 ± 2.46$^{abd}$ | 4.47 ± 0.24$^{abd}$ |
| Fr9 + $CCl_4$ | 60.0(186939*) | 421.33 ± 37.35$^{abc}$ | 430.96 ± 36.92$^{abc}$ | 76.61 ± 4.49$^{abc}$ | 5.22 ± 0.27$^{abc}$ |
| Fr10+ $CCl_4$ | 60.0(379119*) | 597.27 ± 45.74$^{abd}$ | 699.21 ± 48.83$^{abcd}$ | 82.39 ± 3.48$^{abcd}$ | 4.38 ± 0.25$^{abd}$ |

*The doses presented in the tables were normalized with *A. deliciosa* root raw materials; values are expressed as mean ± S.E.M. of six animals in each group. The letters represent statistical significance: $^{a}$ $p < 0.01$, significantly different from the vehicle control; $^{b}$ $p < 0.01$, significantly different from the vehicle+ $CCl_4$; $^{b}$ $p < 0.01$, significantly different from the silymarin+ $CCl_4$; $^{c}$ $p < 0.05$, significantly different from the Fr9 + $CCl_4$. Dunnett's t test was used against the respective control.

393.12 ± 24.62 U/L, decreased 55.64 and 45.17%, compared with that of $CCl_4$ control group hepatotoxicity, respectively which produce relatively higher (in prophylactic studies) and equivalent (in curative studies) effect, compared with silymarin.

## Comparable evaluation of different fractions of Fr3 on biochemical parameters in rats against $CCl_4$-induced hepatic injury (prophylactic study)

In order to identify the hepatoprotective active fraction, five fractions Fr6–Fr10 obtained from the Fr3 were further studied. The results of the studies are summarized in Table 3. The $CCl_4$ administration significantly ($p$ <0.01) increased the level of the rats serum ALT, AST and hepatic lipid peroxidation while significantly ($p$ <0.01) decreased the level of hepatic GSH. A single pretreatment with each fraction of Fr3 shows that all the fractions are effective against acute hepatic damage caused by $CCl_4$. However, of five different extracts (Fr6 - Fr10), Fr9 has relatively higher hepatoprotective effect than other extracts and reference drugs at the dose of 60 mg/kg ($p$<0.05) (Table 3). With treatment of Fr9, the activities of ALT and AST from 1120.68 ± 57.1 U/L and 1042.69 ± 39.56 U/L decreased to 421.33 ± 37.35 and 430.96 ± 36.92 U/L, decrease 62.4% and 58.67%, compared with that of $CCl_4$ control group hepatotoxicity, respectively. The fraction Fr9 was more promising even when compared with silymarin.

The levels of GSH and lipid peroxidation for each group of livers were also determined due to the fact that oxidative stress of tissue generally involves the GSH system and lipid peroxidation. As illustrated in Table 3, the level of lipid peroxidation in the $CCl_4$-induced rat liver decreased using different fraction of Fr3, and the lipid peroxidation was at the minimal level (76.61 ± 4.49 nmol/g liver) when $CCl_4$-induced liver was treated by Fr 9 (Table 3). Moreover, Fr 9 prevented the depletion of hepatic GSH level. When normal liver was treated by $CCl_4$, the GSH level decreased 62.97%, from 6.13 ± 0.22 to 2.27 ± 0.12 µmole/g liver. However, with treatment of 60 mg/kg Fr9, the concentration of GSH only decreased by 14.77% from 6.13 ± 0.22 to 5.22 ± 0.27 µmole/g liver. This value is lower than that of silymarin-treat sample (Table 3).

## Pre- and post-treatment of different dose Fr 9 (TF) against $CCl_4$-induced hepatic injury in rats

The effects of different dose Fr 9 (TF) on $CCl_4$-induced hepatotoxicity in rats were investigated and shown in Tables 4 and 5. Administration of $CCl_4$ alone resulted in a significant increase in normal levels of serum and hepatic parameters (Tables 4 and 5). Pre-treatment with TF (25-200 mg/kg, p.o.) showed different reduction of elevated levels of biochemical parameters ALT, AST, lipid peroxidation, and elevation of decreased level of GSH, in a dose related manner. With increase of the dosage of TF, the activities of ALT, AST and lipid peroxidation decreased, while the level of GSH increased. With 200 mg/kg TF treatment in the $CCl_4$-induced rats, the activities of ALT, AST and lipid peroxidation attained the lowest level of 106.02 ± 27.56 U/L, 149.65 ± 28.93 U/L and 62.69 ± 5.49 (MDA: nmol/g liver), respectively while the level of GSH reached 5.46 ± 0.29 (µmole/g liver). Moreover, ALT, AST and lipid peroxidation decreased by 90.10, 80.69 and 42.11%, while the level of GSH increase 114.12% (Tables 4) compared with that of the $CCl_4$ control group hepatotoxicity, respectively. However, the levels of biochemical parameters ALT, AST, Lipid peroxidation and GSH are not significantly different from the vehicle control by Dunnett's t test (Tables 4).

**Table 4.** Effect of oral administration of active fractions (TF) from ethyl acetate extract (Fr3) against $CCl_4$-induced hepatic injury in rats (prophylactic study).

| Treatment | Dose (mg/kg) p.o. | Serum parameter | | Hepatic parameter | |
|---|---|---|---|---|---|
| | | ALT(U/L) | AST(U/L) | Lipid peroxidation (MDA: nmol/g liver) | Glutathione (µmole/g liver) |
| Vehicle control | – | 84.92 ± 21.20 | 122.48 ± 19.98 | 57.03 ± 3.14 | 5.56 ± 0.24 |
| Vehicle+ $CCl_4$ | – | 1071.42 ± 47.87$^{a}$ | 774.83 ± 49.51$^{a}$ | 108.29 ± 5.59$^{a}$ | 2.55 ± 0.16$^{a}$ |
| TF+ $CCl_4$ | 25.0(77891*) | 568.85 ± 38.24$^{ab}$ | 473.45 ± 46.76$^{ab}$ | 79.66 ± 4.22$^{ab}$ | 3.59 ± 0.25$^{ab}$ |
| TF + $CCl_4$ | 50.0(155782*) | 430.36± 43.63ab | 405.67 ±33.29$^{ab}$ | 76.73 ± 3.03$^{ab}$ | 4.24 ± 0.23$^{ab}$ |
| TF + $CCl_4$ | 100.0(311565*) | 269.47± 28.65ab | 282.26 ± 29.28$^{ab}$ | 70.88 ± 3.56$^{ab}$ | 4.68 ± 0.34$^{ab}$ |
| TF + $CCl_4$ | 200.0(623130*) | 106.02 ± 27.56$^{bNS}$ | 149.65 ± 28.93$^{bNS}$ | 62.69 ± 5.49$^{bNS}$ | 5.46 ± 0.29$^{bNS}$ |

*The doses presented in the tables were normalized with *A. deliciosa* root raw materials; values are expressed as mean ± S.E.M. of six animals in each group. Letters represent statistical significance: $^{a}$ $p < 0.01$, significantly different from the vehicle control; $^{b}$ $p < 0.01$, significantly different from the vehicle+ $CCl_4$; NS: $p > 0.05$, not significantly different from the vehicle control. Dunnett's t test was used against the respective control.

**Table 5.** Effect of oral administration of active fractions (TF) from Ethyl acetate extract (Fr3) against $CCl_4$ induced hepatic injury in rats (curative study).

| Treatment | Dose (mg/kg) p.o. | Serum parameter | | Hepatic parameter | |
|---|---|---|---|---|---|
| | | ALT(U/L) | AST(U/L) | Lipid peroxidation (MDA: nmol/g liver) | Glutathione (µmole/g liver) |
| Vehicle control | – | 93.78 ± 28..27 | 135.32 ± 19.65 | 59.69 ± 4.23 | 5.96 ± 0.28 |
| Vehicle+ $CCl_4$ | – | 1099.49 ± 58.76$^{a}$ | 861.54 ± 62.76$^{a}$ | 117.03± 5.21$^{a}$ | 2.61 ± 0.11$^{a}$ |
| TF+ $CCl_4$ | 25.0(77891*) | 598.05 ± 58.28$^{ab}$ | 506.23 ± 40.65$^{ab}$ | 92.94 ± 4.28$^{ab}$ | 3.28 ± 0.21$^{ab}$ |
| TF + $CCl_4$ | 50.0(155782*) | 498.51± 42.43$^{ab}$ | 458.46 ±41.23$^{ab}$ | 80.72 ± 3.87$^{ab}$ | 4.19 ± 0.19$^{ab}$ |
| TF + $CCl_4$ | 100.0(311565*) | 323.54± 32.87$^{ab}$ | 312.08 ± 36.93$^{ab}$ | 74.64 ± 3.96$^{ab}$ | 4.86 ± 0.32$^{ab}$ |
| TF + $CCl_4$ | 200.0(623130*) | 125.35 ± 28.56$^{bNS}$ | 172.99 ± 58.12$^{bNS}$ | 63.75 ± 4.19$^{bNS}$ | 5.72 ± 0.23$^{bNS}$ |

*The doses presented in the tables were normalized with *A. deliciosa* root raw materials; values are expressed as mean ± S.E.M. of six animals in each group. Letters represent statistical significance: $^{a}$ $p < 0.01$, significantly different from the vehicle control; $^{b}$ $p < 0.01$, significantly different from the vehicle+ $CCl_4$; NS: $p > 0.05$, not significantly different from the vehicle control. Dunnett's t test was used against the respective control.

Post-treatment with TF also showed significant reversal of these elevated levels in a dose-related manner for ALT, AST, lipid peroxidation and GSH (Tables 5). When at the dose of 200 mg/kg TF was used in treating the $CCl_4$-induced rat. The activities of ALT, AST and lipid peroxidation attained low levels, decreasing by 88.60, 79.92 and 45.53%, while the level of GSH increased by 147.62% respectively compared with that of the $CCl_4$ control group hepatotoxicity (Tables 5). Moreover, the levels of biochemical parameters ALT, AST, Lipid peroxidation and GSH were not significantly different from the vehicle control by Dunnett's t test (Tables 5).

## Identification of the main constituents of TF

Compound I (Figure 1) was obtained as a white amorphous powder, and it gave a positive coloration with the Liebermann-Burchard test for triterpenoids. Mp 291~292°C. IR $v_{max}$($cm^{-1}$): 3429, 2926, 1685, 1450, indicate the presence of the hydroxyl ($v_{max}$ 3429 $cm^{-1}$) and carboxy ($v_{max}$ 1685 $cm^{-1}$). EI-MS showed a molecular ion peak at (*m/z*): 456($M^+$), compatible with the molecular formula $C_{30}H_{48}O_3$. This compound was compared with the authentic sample on TLC, and it was observed that they had the same $R_f$ values. For $^1$H-NMR spectroscopy: $\delta$ 0.65 (3H, *s*, $CH_3$), 0.68 (3H, *s*, $CH_3$), 1.02 (3H, *s*, $CH_3$), 1.22 (3H, *s*, $CH_3$), 1.44 (3H, *s*, $CH_3$), 0.90 (6H, *s*, $2CH_3$), 3.30~3.40 (br.,1H), 4.10 (br.,1H), 5.08 (S, 1H). The results of $^{13}$CNMR are shown in Table 6; the spectral data are in agreement with those reported in the literature (Srivasta and Jain, 1989; Gong, 1986).

Compound II (Figure 1) was obtained as a white powder, and it gave a positive coloration with the Liebermann-Burchard test for triterpenoids. Mp 308~310°C. IR $v_{max}$($cm^{-1}$): 3444.14, 2923.07, 2852.90, 1689.18, 1462.26, 1376.90,1033.79. EI-MS showed a molecular ion peak at (*m/z*): 487.7($M^+$) compatible with the molecular formula $C_{30}H_{48}O_5$. For the $^1$H-NMR: $\delta$: 5.45(1H, *s*, H-12, 4.26(1H, *m*, 10.8, H-2β), 4.20(1H, *d*, H-3β), 3.90 and 3.72(2H, AB *d*, 11.5 H2-23), 2.59(1H, *d*, 11.2, H-18), 1.27, 1.1 O, 1.04, 0.90, 0.93 and 0.85 (18H,

**Table 6.** $^{13}C$ NMR spectral data of compounds I, II and III (δc).

| No. of carbon (C) | Compound I ($C_5D_5N$) | Compound II ($C_5D_5N$) | Compound III ($C_5D_5N$) | No. of carbon (C) | Compound I ($C_5D_5N$) | Compound II ($C_5D_5N$) | Compound III ($C_5D_5N$) |
|---|---|---|---|---|---|---|---|
| 1 | 39.20 | 43.4 | 43.3 | 16 | 25.10 | 25.1 | 26.7 |
| 2 | 28.30 | 66.4 | 66.4 | 17 | 48.20 | 48.3 | 48.2 |
| 3 | 78.30 | 74.4 | 74.4 | 18 | 53.70 | 53.8 | 54.9 |
| 4 | 37.40 | 45.3 | 43.5 | 19 | 39.60 | 39.7 | 30.1 |
| 5 | 56.00 | 49.4 | 49.7 | 20 | 39.50 | 39.6 | 38.7 |
| 6 | 18.90 | 19.1 | 19.2 | 21 | 31.20 | 31.4 | 35.5 |
| 7 | 33.70 | 34.2 | 34.2 | 22 | 37.60 | 37.7 | 38.3 |
| 8 | 39.50 | 40.3 | 40.3 | 23 | 28.90 | 65.4 | 65.4 |
| 9 | 48.20 | 48.3 | 48.1 | 24 | 15.80 | 17.3 | 14.2 |
| 10 | 42.60 | 38.7 | 38.2 | 25 | 16.70 | 23.9 | 17.1 |
| 11 | 23.80 | 24.0 | 24.5 | 26 | 17.60 | 21.6 | 16.6 |
| 12 | 125.80 | 125.6 | 127.3 | 27 | 24.30 | 23.9 | 24.5 |
| 13 | 139.40 | 139.6 | 139.6 | 28 | 180.00 | 180.0 | 180.5 |
| 14 | 40.10 | 42.7 | 42.2 | 29 | 17.60 | 19.0 | 27.0 |

6×Me). The results of $^{13}C$ NMR are shown in Table 6; the spectral data are in agreement with those reported in the literature (Gong, 1986, Lai and Xu, 2007).

Compound III (Figure 1) was obtained as a white powder, and it gave a positive coloration with the Liebermann-Burchard test for triterpenoids. Mp 312~315°C. IR $v_{max}$($cm^{-1}$): 3420.17, 2923.35, 2851.47, 1691.77, 1629.58, 1572.78, 1454.69, 1386.43, 1045.29. EI-MS showed a molecular ion peak at (*m/z*): 503.7($M^+$), compatible with the molecular formula $C_{30}H_{48}O_6$. For the $^1H$-NMR: δ: 0. 83(3H, *d*, *J*=6 Hz, C30-Me), 0.72, 0.91, 1.09, 1.15, 1.25 (each 3 H, *s*, 5×Me), 2.40(1H, brs, C18β-H ), 2.45(1H, *d*t, *J*=13, 4.5 Hz, C5α-H), 2.96(1H, d, *J*=10.5 Hz , C3α-H ), 3.31(1H, d, *J*=11. 5 Hz, C24α-H), 3.72(1 H, dt, *J*=1.05, 4 Hz, C2α-H), 3. 94(1H, d, *J*=11.5 Hz, C24β-H), 5.18(1H, brt, *J*=4 Hz, C12-H). The results of $^{13}C$ NMR are shown Table 6; the spectral data are in agreement with those reported in the literature (Gong, 1986, Lai and Xu, 2007).

In the present study, the in *vivo* hepatoprotective activity of different extracts of *A. deliciosa* root was assessed by monitoring different serum and hepatic biochemical parameter status using an active fraction (TF), which was obtain from the activity directed fractionation of aqueous alcoholic extract of *A. deliciosa* root, against $CCl_4$-induced hepatotoxicity (Tables 2 to 5). Oral administration of TF showed significant hepato-protective activity both in preventive and curative treatments against $CCl_4$-induced hepatic damage (Tables 4 and 5), and subsequent recovery towards normalization of these enzymes at a dose 200 mg/kg. The leakage of hepatic housekeeping enzymes such as ALT, AST is commonly used as an indirect biochemical index of hepatocellular damage (Recknagel and Lombardi, 1961; Klaassen and Watkin, 1984). The extent of hepato-protection afforded by the extracts and TF against the chemically induced hepatic injury can be compared on the basis of serum and hepatic parameters (Tables 3 to 5).

It is well established that the pathogenesis of $CCl_4$-induced hepatotoxicity associated with the substantial increase in lipid per-oxidation as was evident from elevated MDA level in liver homogenate with a concurrent fall in hepatic GSH content, which was very much supportive with the strong hepatotoxic response of these experimental models (Klaassen and Watkin, 1984; Burk, 1983). Treatment with TF significantly reversed and restored these altered level of these parameters to normal (Tables 4 and 5). The studied result suggests that oral administration of TF has a beneficial effect on the hepato-protection as observed in the present study. However, based on the results illustrated in the present study, it may be suggested that the use of TF for treatment of chemical, drug, viral or pollutant mediated hepatic ailments is well justified as it has inhibited the hepatocellular metabolic alterations that are mediated by $CCl_4$ treatments. These findings confirm its reported therapeutic value in traditional system of medicine and dispersal by herbal dealers in liver ailments. Further studies using some more models of experimental injury may help to establish a definite rationale for its therapeutic exploration as a potent hepatoprotective drug.

## Conclusion

From the overall results, it can be concluded that the TF exhibited high hepatoprotective activities *in vivo* in a dose-related manner as indicated by the action against

$CCl_4$ toxicity. The hepatoprotective action may be mainly mediated by the enhancement of hepatic glutathione regeneration capacity and decreased level of lipid peroxidation, particularly under conditions of $CCl_4$-induced oxidative stress. The main chemical constituents of TF are three triterpenoids, namely 3β-hydroxy-urs-12-en-28-oic acid (I); 2α, 3α, 23-trihydroxy-urs-12-en-28-oic acid (II); 2α, 3β, 19, 23- tetrahydroxy-urs-12-en-28-oic acid (III).

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