

# A New Octadecenoic Acid Derivative from *Caesalpinia gilliesii* Flowers with Potent Hepatoprotective Activity

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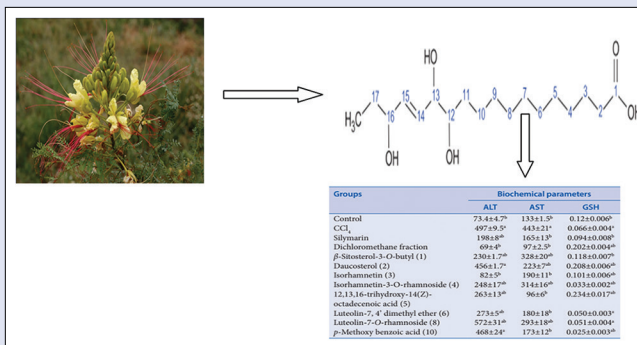
## ABSTRACT

**Background:** *Caesalpinia gilliesii* Hook is an ornamental shrub with showy yellow flowers. It was used in folk medicine due to its contents of different classes of secondary metabolites. In our previous study, dichloromethane extract of *C. gilliesii* flowers showed a good antioxidant activity. **Aim of the Study:** Isolation and identification of bioactive hepatoprotective compounds from *C. gilliesii* flowers dichloromethane fraction. **Materials and Methods:** The hepatoprotective activity of dichloromethane fraction and isolated compounds were studied in CCl<sub>4</sub>-intoxicated rat liver slices by measuring liver injury markers (alanine aminotransferase, aspartate aminotransferase and glutathione [GSH]). All compounds were structurally elucidated on the basis of electron ionization-mass spectrometry, one- and two-dimensional nuclear magnetic resonance. **Results:** A new 12,13,16-trihydroxy-14(Z)-octadecenoic acid was identified in addition to the known  $\beta$ -sitosterol-3-O-butyl, daucosterol, isorhamnetin, isorhamnetin-3-O-rhamnoside, luteolin-7,4'-dimethyl ether, genistein-5-methyl ether, luteolin-7-O-rhamnoside, isovanillic acid, and p-methoxybenzoic acid. Dichloromethane fraction and isorhamnetin were able to significantly protect the liver against intoxication. Moreover, the dichloromethane fraction and the isolated phytosterols induced GSH above the normal level. **Conclusion:** The hepatoprotective activity of *C. gilliesii* may be attributed to its high content of phytosterols and phenolic compounds.

**Key words:** *Caesalpinia*, fatty acid, hepatoprotective activity, isorhamnetin, liver injury markers (alanine aminotransferase, aspartate aminotransferase and glutathione), octadecenoic acid, plant phenolics

## SUMMARY

- Bioactive Hepatoprotective phytosterols and phenolics from chloroform extract of *Caesalpinia gilliesii*



**Abbreviations used:** ALT: Alanine Aminotransferase; AST: Aspartate aminotransferase; GSH: Glutathione; SC50: Scavenging Capacity 50 (SC 50); COSY: Correlation spectroscopy; NMR: Nuclear Magnetic Resonance; CC: Column chromatography; EI-MS: Electron-impact mass spectrometry; HSQC: Heteronuclear single-quantum correlation.

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## INTRODUCTION

Liver cell injury through various toxic chemicals, excessive alcohol consumption, and viral infections is well documented. A potential liver damage is elicited by complex mechanisms involving free radicals inducing oxidative stress which can lead to peroxidation, DNA damage, and inflammation.<sup>[1]</sup> The hepatotoxicity of CCl<sub>4</sub> is due to the metabolic formation of the highly reactive trichloromethyl radical which attacks polyunsaturated fatty acids of the endoplasmic reticulum membrane resulting in a loss of cytochrome p<sub>450</sub> leading to its functional failure with a decrease in protein modification and accumulation of triglycerides.<sup>[2]</sup> Antioxidants, which mop up free radicals, can reduce that process. Around 170 phytoconstituents isolated from 110 plants have been reported to possess strong antioxidant<sup>[3]</sup> and hepatoprotective activities such as *Silybum marianum*,<sup>[4]</sup> *Tridax procumbens*,<sup>[5]</sup> *Strychnos potatorum*,<sup>[6]</sup> and *Andrographis paniculata*.<sup>[7]</sup>

The *Caesalpinioideae* (family Fabaceae) represent approximately 11% of all legume taxa with more than 2250 mostly tropical and subtropical trees and shrubs. The genus *Caesalpinia*, which comprises more than forty mostly tropical trees and shrubs, are widely used in

folk medicine.<sup>[8]</sup> For example, *Caesalpinia bonducella* seeds eliminate the symptoms of diabetes mellitus.<sup>[9]</sup> Genus *Caesalpinia* was also reported to possess anticancer,<sup>[10]</sup> antioxidant, and hepatoprotective properties.<sup>[11]</sup> *Caesalpinia gilliesii* Hook (known as Yellow Bird of Paradise) is an ornamental shrub with showy yellow flowers [Figure 1]. The plant is native to Argentina but has been cultivated worldwide. *Caesalpinia* was reported to contain different classes of secondary metabolites include terpenoids, flavonoids, and phenolics.<sup>[8,12,13]</sup> Since

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**Figure 1:** *Caesalpinia gilliesii* flowers (x = 1/4)

oxidative stress is one of the main causes of liver toxicity, agents with the ability to protect the liver against reactive pro-oxidant species may be therapeutically useful. This is true for several polyhydroxylated flavonoids, which have already been shown to be hepatoprotective, as in the case of catechins<sup>[14]</sup> and quercetin.<sup>[15]</sup> Dihydrobonducellin and 2-methoxy dihydro-bonducellin, isolated from *Caesalpinia pulcherrima*, showed radical scavenging activity that were better than the positive antioxidants controls with  $SC_{50}$  values of 352  $\mu$ M and 325  $\mu$ M, respectively, compared with vitamin C ( $SC_{50}$  = 852  $\mu$ M).<sup>[16]</sup>

However, the dichloromethane extract of *C. gilliesii* flowers showed antioxidant activity ( $SC_{50}$  = 45.5  $\mu$ g/mL comparable to standard rutin  $SC_{50}$ : 24  $\mu$ g/ml).<sup>[11]</sup> In this context and in continuation of our previous work on *C. gilliesii*,<sup>[17]</sup> this study was designed to evaluate the *in vitro* hepatoprotective activity of the dichloromethane fraction of *C. gilliesii* flowers. In addition, a detailed phytochemical investigation of that fraction was carried out to isolate and identify its bioactive compounds. The hepatoprotective activity of the isolated compounds was also evaluated.

## MATERIALS AND METHODS

### General experimental procedures

<sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were measured on a Varian 300 MHz and Bruker 400 MHz AC NMR spectrometers. Based on their solubility, samples were dissolved in different deuterated solvents Deutero<sup>®</sup> (Kastellaun, Germany). Electron ionization-mass spectrometry (EI-MS) spectra were recorded on Thermo Scientific, Trace gas chromatograph Ultra coupled with ISQ Single Quadruple MS Capillary column (National Research Center, Egypt). Two-dimensional (2D) NMR experiments (double quantum filter correlated spectroscopy [COSY], heteronuclear single-quantum correlation spectroscopy [HSQC]) were carried out with all isolated compounds using the pulse sequences from the Varian and Bruker user library. On the basis of 2D-NMR analyses, assignments of <sup>1</sup>H and <sup>13</sup>C signals were established. Column chromatography (CC) was carried out on silica gel 60 (0.063–0.2 mm) (Sigma-Aldrich Co., USA) and Sephadex LH-20 (25–100 mm) (Sigma-Aldrich Co., USA) using mixtures of dichloromethane and methanol with different ratios for silica gel 60 and 100% methanol for Sephadex LH-20. Fractions were monitored by thin layer chromatography on precoated silica gel 60  $F_{254}$  (0.25 mm) (Merck<sup>®</sup>, Darmstadt, Germany) using either *p*-anisaldehyde/sulfuric acid spray reagent and heating at 100°C for 7 min,  $NH_3$  or 5%  $AlCl_3$  reagent.<sup>[18]</sup>

### Plant material

*C. gilliesii* flowers were collected from Borg El Arab (Egypt) in May 2013 [Figure 1]. The plant identity was kindly confirmed by Prof. Abd El-halem A. El-Meged (Agriculture Research Center, Cairo, Egypt). A voucher specimen (CP# 1203) is kept at the Department of Pharmacognosy, Faculty of Pharmacy, October 6 University.

### Chemicals

Chemicals and solvents were of analytical grade unless mentioned and were purchased from Merck<sup>®</sup> (Darmstadt, Germany), J.T. Backer<sup>®</sup> (Deventer, The Netherlands) and Theo Seulberger<sup>®</sup> (Karlsruhe, Germany). Media and supplements for cell cultures were obtained from Gibco<sup>®</sup> (Karlsruhe, Germany) and Greiner Labortechnik<sup>®</sup> (Frickenhausen, Germany).

### Extraction and isolation

Freshly collected plant flowers were washed 3 times with running tap water and then with distilled water followed by drying in shade for 8 days. A hydroalcoholic (70%) extract of flowers was prepared by powdering 500 g then defatted by *n*-hexane (2 L  $\times$  3 times). The solvent was filtered, and cake was exhaustively macerated with 70% ethanol. Ethanol was filtered and evaporated under reduced pressure yielding 60 g (12%) of hydroalcoholic extract. Subsequently, 55 g dried *C. gilliesii* flower extract was suspended in 500 ml distilled water and sonicated for 30 min. The extract was fractionated using solvent–solvent extraction with *n*-hexane (6  $\times$  1 L), dichloromethane (6  $\times$  1 L). The resulting fractions were then dried under reduced pressure in a rotary evaporator (Sinco Technology Co. Ltd, Shanghai, China).

The dichloromethane residue (1.5 g) was subjected to silica gel CC (2 cm  $\times$  50 cm, 120 g) using  $CH_2Cl_2:CH_3OH$  as eluents with different ratios in increasing polarity manner to give six fractions (I–VI). In our previous work,<sup>[17]</sup> Fraction I (100 mg) that eluted with  $CH_2Cl_2$  (100%) was purified to give compound 1 (20 mg). However, Fraction II (200 mg) that eluted with  $CH_2Cl_2:CH_3OH$  (90:10) gave compound 2 (20 mg) after being further separated on silica gel CC. Fraction III (450 mg) that eluted with  $CH_2Cl_2:CH_3OH$  (75:25) was further chromatographed on silica gel CC with the same mobile phase to give subfractions 1–15. Subfractions 1 and 2 were pure compounds 3, 4 (25 and 15 mg, respectively). In continuation; subfractions 3–7 of that fraction were collected together afforded compound 5 (30 mg). Subfractions 10–15 were collected together afforded compound 6 (55 mg). Fraction IV (50 mg) that eluted with  $CH_2Cl_2:CH_3OH$  (50:50) was further purified afforded compound 7 (5 mg). Fraction V (200 mg) that eluted with  $CH_2Cl_2:CH_3OH$  (25:75), was further chromatographed on silica gel CC with the same mobile phase afforded compound 8 (40 mg). Fraction VI (400 mg) that eluted with 100%  $CH_3OH$  was further chromatographed on Sephadex LH 20 CC afforded compound 9 and 10 (5 mg and 30 mg, respectively). All isolated compounds were evaporated under reduced pressure and subjected to further purification on Sephadex LH-20 CC (1 cm  $\times$  40 cm, 15 g) using 100% methanol as mobile phase before being analyzed.

### Compound 5

Rosette crystals sprayed with *p*-anisaldehyde to give a dark blue color indicative for its phytosterol nature. EI-MS (*m/z*) 330 [M], 312 [M–H<sub>2</sub>O], 294 [M–2 H<sub>2</sub>O], 287 [M–CO<sub>2</sub>]<sup>+</sup>, 276 [M–3H<sub>2</sub>O]<sup>+</sup>, 244 [M–C<sub>5</sub>H<sub>10</sub>O], 184 [M–C<sub>7</sub>H<sub>14</sub>O<sub>3</sub>], 147 [M–C<sub>11</sub>H<sub>22</sub>O<sub>2</sub>]<sup>+</sup>, and 86 [M–C<sub>13</sub>H<sub>24</sub>O<sub>4</sub>]. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$  (ppm), *J* (Hz) 7.04 (*s*, OH), 5.69 (2H, *t*, *J* = 8 Hz, H-14, 15), 4.05 (1H, *m*, H-16), 3.89 (1H, *t*, *J* = 8 Hz, H-13), 3.40 (1H, *m*, H-12), 2.26 (2H, *t*, *J* = 8 Hz, H-2), 1.60–1.50 (6H, *m*, H-3, 11, 17), 1.31 (14H, remaining H-4, 5, 6, 7, 8, 9, 10), 0.89 (3H, *t*, *J* = 8 Hz, H-18).

$^{13}\text{C}$  NMR DEPT Q (100 MHz,  $\text{CD}_3\text{OD}$ ) 177.71 (C = O), 34.95 ( $\text{CH}_2$ -2), 23.73 ( $\text{CH}_2$ -3), 26.83 ( $\text{CH}_2$ -4), 30.21 ( $\text{CH}_2$ -5), 30.38 ( $\text{CH}_2$ -6), 30.64 ( $\text{CH}_2$ -7), 33.00 ( $\text{CH}_2$ -8), 136.52 (CH-15), 130.94 (CH-14), 38.32 ( $\text{CH}_2$ -11), 73.07 (CH-12), 75.76 (CH-13), 26.24 ( $\text{CH}_2$ -9), 33.51 ( $\text{CH}_2$ -10), 76.53 (CH-16), 26.08 ( $\text{CH}_2$ -17), 14.41 ( $\text{CH}_3$ -18).

## Hepatoprotective protocol

Hepatoprotective assays were carried out following the protocols developed by Wormser.<sup>[19]</sup> After cervical dislocation of the normal rats, liver lobes were removed and transferred to prewarmed Krebs Ringer Hepes (KRH) (Hepes 2.5 mM pH 7.4, NaCl 118 mM, KCl 2.85 mM,  $\text{CaCl}_2$  2.5 mM,  $\text{KH}_2\text{PO}_4$  1.5 mM,  $\text{MgSO}_4$  1.18 mM, and glucose 4.0 mM). The liver was then cut into thin slices using sharp scalpel blades. The slices were weighed, and slices weighing between 4 and 6 mg were used for the experiment. Each experiment consisted of 20–22 slices weighing together 100–120 mg. These slices were washed with 10 ml KRH medium, every 10 min over a period of 1 h. These were then preincubated for 60 min in small plugged beakers containing 2 ml KRH on a shaker water bath at 37°C. After incubation period, the slices were divided into groups according to the experimental design; control group were left in the medium without any additives,  $\text{CCl}_4$  group was treated with  $\text{CCl}_4$  (15.5 mM) alone, the silymarin group plus  $\text{CCl}_4$  (15.5 mM) and silymarin (100  $\mu\text{g}/\text{ml}$ ), other experimental groups contained  $\text{CCl}_4$  (15.5 mM) plus examined fraction or isolated compounds (100  $\mu\text{g}/\text{ml}$ ). All the groups were incubated at 37°C for 24 h., and then the supernatant of each group was collected separately and kept at  $-20^\circ\text{C}$  till further investigations.

## Biochemical assays

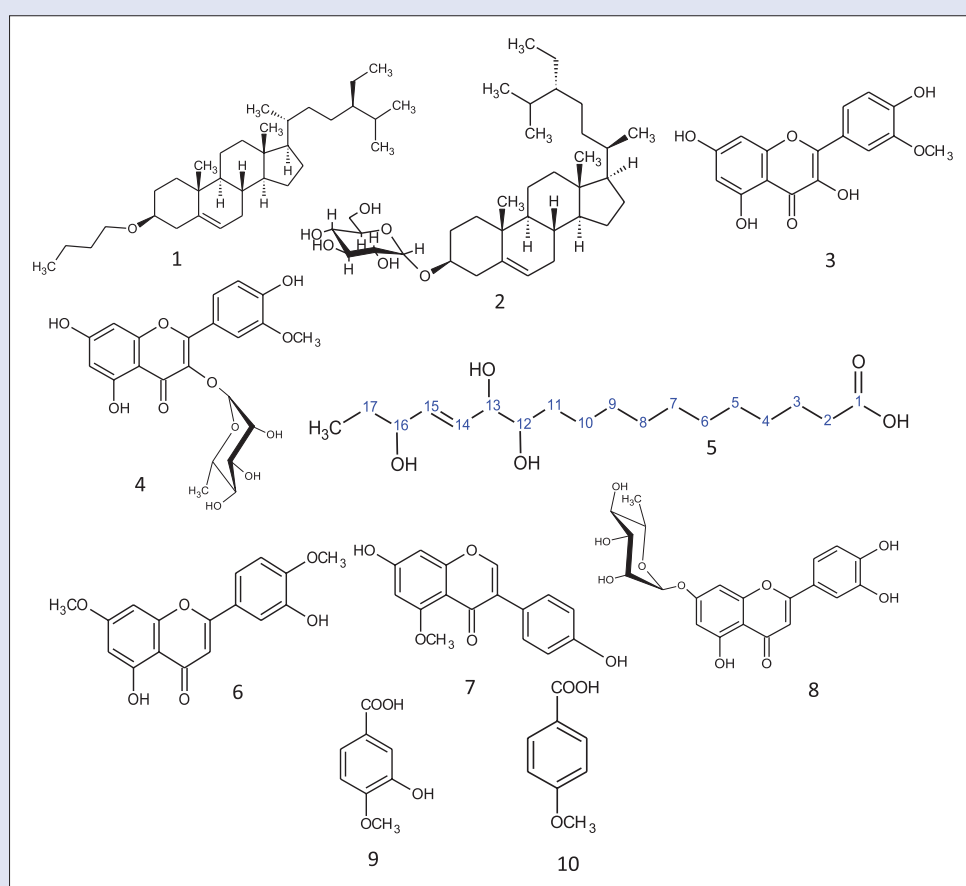
Reduced glutathione (GSH) is the major intracellular low-molecular-weight thiol that plays a critical role in the cellular defense against oxidative stress in mammalian cells. BioVision's ApoGSH™ Glutathione Colorimetric Assay Kit provides a convenient colorimetric method for analyzing either total glutathione or the reduced form glutathione alone using a microtiter plate reader. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum concentration is elevated in hepatic disease associated with necrosis, cholestasis, cirrhosis, liver carcinoma, and after administration of various drugs. The activities of serum AST and ALT were assayed according to Reitman and Frankel.<sup>[20]</sup>

## Data analysis

All biological experiments were repeated at least 6 times. Data are presented as mean  $\pm$  standard error of the mean. The  $\text{IC}_{50}$  values were calculated using a four parameter logistic curve (SigmaPlot1 11.0, Systat Software, Inc., Richmond, California, USA) and data were drawn and statistically analyzed using Student's *t*-test using GraphPad Prism1 5.0 (GraphPad Prism Software, Inc., San Diego, USA). The criterion for statistical significance was taken as  $P < 0.05$ .

## RESULTS AND DISCUSSION

Our previous study had resulted in the isolation of four compounds  $\beta$ -sitosterol-3-*O*-butyl (1), daucosterol (2), isorhamnetin (3), and isorhamnetin-3-*O*-rhamnoside (4).<sup>[17]</sup> A consecutive phytochemical



**Figure 2:** 1) 3-*O*-butyl-  $\beta$ - sitosterol, 2) daucosterol, 3) isorhamnetin, 4) isorhamnetin-3-*O*-rhamnoside, 5) 12,13,16-trihydroxy-14(*Z*)-octadecenoic acid, 6) 5,3'-dihydroxy-7,4'- dimethoxyflavone, 7) genistein-5-methyl ether, 8) Luteolin-7-*O*-rhamnoside, 9) Isovanillic acid and 10) *p*- methoxy benzoic acid



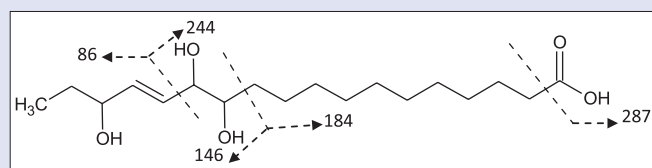
investigation of the dichloromethane fraction from flowers using CC resulted in the isolation of a new 12,13,16-trihydroxy-14(Z)-octadecenoic acid (5), furthermore luteolin-7,4'-dimethyl ether (6), genistein-5-methyl ether (7), luteolin-7-O-rhamnoside (8), isovanillic acid (9), and *p*-methoxy benzoic acid (10) were isolated [Figure 2]. Identification of all known compounds was achieved by comparing their spectral data with that published.<sup>[21,22]</sup>

Compound (5) gives a dark blue color with *p*-anisaldehyde indicative for its phytosterol nature. EI-MS spectral analysis [Figure 3] showed a molecular weight of  $m/z$  330 [ $C_{18}H_{34}O_5$ ] and illustrated by  $^{13}C$  NMR DEPT Q spectrum of 18 carbon signals accounting for 1  $CH_3$ , 11  $CH_2$ , 5  $CH$  groups and 1 quaternary carbon atoms. Other molecular ions peaks at  $m/z$  312 [ $M-H_2O$ ], 294 [ $M-2 H_2O$ ], and 277 [ $M-3 H_2O$ ]<sup>+</sup> suggested for trihydroxy groups<sup>[23]</sup> that confirmed by three downfield proton signals at  $\delta$  (ppm) 4.05 (1H, *m*), 3.89 (1H, *t*,  $J = 8$  Hz), 3.40 (1H, *m*) and appeared at 73.07, 75.76, and 76.53 in  $^{13}C$  NMR DEPT Q meaning three CH-oxygenated carbons. Other  $m/z$  287 [ $M-CO_2$ ]<sup>+</sup> revealed the presence of carboxyl moiety illustrated by one signal at 177.71 ppm in  $^{13}C$  DEPT Q spectra. *Cis* olefinic CH carbons appeared in  $^1H$  NMR spectrum at  $\delta$  (ppm) 5.69 (2H, *t*,  $J = 8$  Hz) and at 136.52 130.94 in  $^{13}C$  DEPT Q spectra.<sup>[24]</sup> It showed also signal at 2.26 (2H, *t*,  $J = 8$  Hz) characteristic for  $\alpha$   $CH_2$  of carboxyl group, signals at 1.50–1.60 (6H, *m*, 3 $CH_2$ ) were assigned to be 1  $\beta$   $CH_2$  of carboxyl group and 2 other  $\alpha$   $CH_2$  of oxygenated carbon.<sup>[25]</sup> Signals at 1.31 (14 H) and appeared from 38.32–23.73 in  $^{13}C$  DEPT Q spectra were for 11  $CH_2$  carbons.<sup>[26]</sup> Signal at 0.89 (3H, *t*,  $J = 8$  Hz) and 14.41 in  $^1H$  NMR and  $^{13}C$  DEPT Q spectra, respectively, were assigned for terminal methyl group. The hydroxyl and unsaturation positions were assigned based on their correlations in COSY and HSQC spectrum. Hence, compound 5 was identified to be the new natural product 12,13,16-trihydroxy-14(Z)-octadecenoic acid.

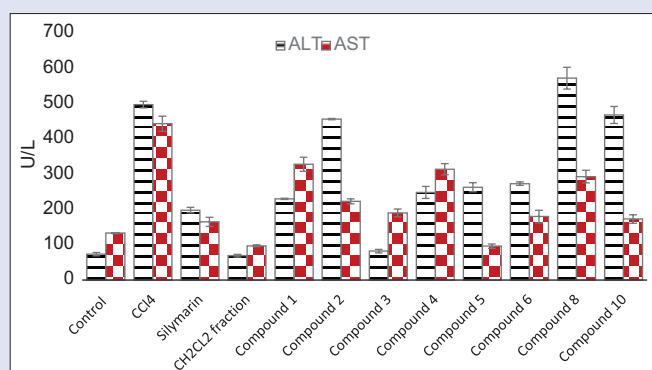
The liver is involved in almost all the biochemical pathways, and it functions as a center of metabolism of carbohydrates, proteins, lipids, and excretion of waste metabolites. Silymarin, a flavonolignan mixture from *S. marianum*, is used as standard hepatoprotective compound since it was reported to have a protective effect on the plasma membrane of the hepatocytes, and clinical studies have shown its efficacy in patients with Amanita poisoning and liver cirrhosis.<sup>[2]</sup> The hepatotoxicity of  $CCl_4$  is due to its active metabolite, trichloromethyl radical, which attacks macromolecules inducing degradation of biomembranes of the endoplasmic reticulum.<sup>[2]</sup> Liver injury parameters including ALT and AST were studied, which reflect the condition of hepatocytes and inflammatory destruction. A significant ( $P < 0.0001$ ) elevation of the biochemical parameters ALT and AST in the  $CCl_4$  group ( $497 \pm 9$ ,  $443 \pm 21$  U/L, respectively) compared to the control group ( $73.4 \pm 4.7$ ,  $133 \pm 1.5$  U/L, respectively) was detected. Liver slices (hepatocytes) that were treated with standard silymarin were protected considerably against the toxicity indicated by the level of the biochemical parameters ( $198 \pm 8$ ,  $165 \pm 13$  U/L, respectively) comparable to  $CCl_4$  group ( $P < 0.001$ ). The enzyme levels in liver slices treated with the dichloromethane fraction ( $69 \pm 4$ ,  $97 \pm 2.5$  U/L, respectively) were similar to that of the control and silymarin group ( $P > 0.05$ ) [Table 1].

Isorhamnetin showed a significant protection as compared to  $CCl_4$  group ( $P < 0.001$ ) and nonsignificant to control group. Our previous study<sup>[17]</sup> had suggested that because isorhamnetin has potent antioxidant activity, it might be promising as hepatoprotective agent. In our present study, we found that the sugar affects that activity as glycosides residue has a low influence on hepatoprotective activity. Furthermore, compounds 1 and 2 exhibited a significant reduction for ALT and AST as compared to  $CCl_4$  group ( $P < 0.001$ ) but less active than isorhamnetin [Figure 4].

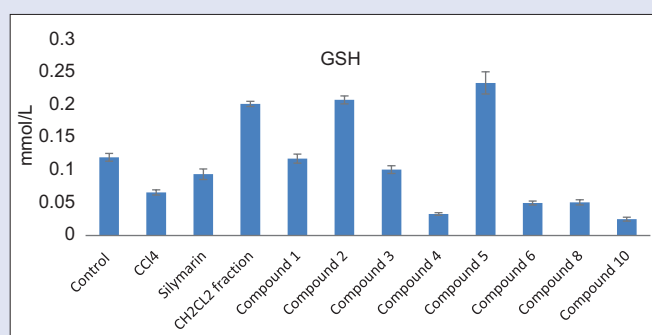
The GSH level was decreased in the  $CCl_4$  group ( $0.066 \pm 0.004$  mmol/L), which reached about half its value as compared to control group ( $0.12 \pm 0.006$  mmol/L). Liver slices that were treated with standard silymarin were protected considerably against reduction in GSH level ( $0.094 \pm 0.008$  mmol/L) when compared with the  $CCl_4$  group ( $P < 0.001$ ). Treatment with the dichloromethane fraction significantly increased GSH above its normal level ( $0.202 \pm 0.004$  mmol/L) ( $P < 0.001$ ). The isolated new compound 5 and compound 2 also increased GSH above its normal level ( $0.234 \pm 0.017$ ,  $0.208 \pm 0.006$  mmol/L, respectively) ( $P < 0.001$ ) [Figure 5]. It is likely that the phytosterols (compound 5 and 2) interact with the biomembranes by forming a complex with cholesterol<sup>[27]</sup> while flavonoids, in general, are nonselective inhibitors of many enzymes and other proteins. Under normal physiological conditions, the polyphenols dissociate into negatively charged phenolate ions, which can interact with positively



**Figure 3:** Mass fragmentation of 12, 13, and 16-trihydroxy-14(Z)-octadecenoic acid



**Figure 4:** Hepatoprotective effects of the dichloromethane fraction of *C. gilliesii* flowers and its isolated compounds indicated by ALT and AST (U/L) activities (values are mean  $\pm$  SEM of 6 replicates)



**Figure 5:** Effect of the dichloromethane fraction of *C. gilliesii* flowers and its isolated compounds on reduced glutathione (GSH mmol/L) of  $CCl_4$ -intoxicated rats hepatocytes (values are mean  $\pm$  SEM of 6 replicates)

**Table 1:** Effect of the dichloromethane fraction of *C. gilliesii* flowers and its isolated compounds on biochemical parameters of CCl<sub>4</sub>-intoxicated rat liver slices (values are mean±SEM of 6 replicates)

Groups	Biochemical parameters		
	ALT	AST	GSH
Control	73.4±4.7 <sup>b</sup>	133±1.5 <sup>b</sup>	0.12±0.006 <sup>b</sup>
CCl <sub>4</sub>	497±9.5 <sup>a</sup>	443±21 <sup>a</sup>	0.066±0.004 <sup>a</sup>
Silymarin	198±8 <sup>ab</sup>	165±13 <sup>b</sup>	0.094±0.008 <sup>b</sup>
Dichloromethane fraction	69±4 <sup>b</sup>	97±2.5 <sup>b</sup>	0.202±0.004 <sup>ab</sup>
β-Sitosterol-3-O-butyl (1)	230±1.7 <sup>ab</sup>	328±20 <sup>ab</sup>	0.118±0.007 <sup>b</sup>
Daucosterol (2)	456±1.7 <sup>a</sup>	223±7 <sup>ab</sup>	0.208±0.006 <sup>ab</sup>
Isorhamnetin (3)	82±5 <sup>b</sup>	190±11 <sup>b</sup>	0.101±0.006 <sup>ab</sup>
Isorhamnetin-3-O-rhamnoside (4)	248±17 <sup>ab</sup>	314±16 <sup>ab</sup>	0.033±0.002 <sup>ab</sup>
12,13,16-trihydroxy-14(Z)-octadecenoic acid (5)	263±13 <sup>ab</sup>	96±6 <sup>b</sup>	0.234±0.017 <sup>ab</sup>
Luteolin7, 4' dimethyl ether (6)	273±5 <sup>ab</sup>	180±18 <sup>b</sup>	0.050±0.003 <sup>a</sup>
Luteolin7-O-rhamnoside (8)	572±31 <sup>ab</sup>	293±18 <sup>ab</sup>	0.051±0.004 <sup>a</sup>
p-Methoxy benzoic acid (10)	468±24 <sup>a</sup>	173±12 <sup>b</sup>	0.025±0.003 <sup>ab</sup>

<sup>a</sup>Statistical significance in comparison to control group  $P < 0.001$ . <sup>b</sup>Statistical significance in comparison to CCl<sub>4</sub> group  $P < 0.001$

charged function groups of different enzymes and proteins in the cell through ionic and hydrogen bonds, which dramatically inhibit their 3D structure and, in consequence, their functions.<sup>[27]</sup> These properties might explain the hepatoprotective effects seen in this study. Based on these results, we conclude that the hepatoprotective activity of *C. gilliesii* may be attributed to its high content of phytosterols and phenolic compounds.<sup>[28]</sup> It may become a lead in the future for the development of hepatoprotective drug. However, animal experiments must still show its efficacy under *in vivo* conditions.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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### Research Scopes:

- Active natural products biotechnology
- Molecular evolution

### Bibliometrics (March 2016):

**Google Scholar:** h-index = 70 (h = 44 since 2010), 24190 total citations

**Web of Knowledge:** 655 ISI-indexed publications; total number of citations = 12750; H-Index = 50

**Research Gate:** 804 publications; 1194 Impact points, R6Score 48.30, 35.500 reads