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CHROMATOGRAPHIC ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF SOME CHEMICAL CONSTITUENTS OF PETROSELINUM CRISPUM LEAVES AND EVALUATING AS HEPATOPROTECTIVE AGENTS

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Petroselinum crispum Mill. (family Apiaceae) is widely used as an essential spice due to its health benefits. This study aimed to investigate the constituents of P.crispum aerial parts and evaluate its hepatoprotective activity. Ethyl acetate and n-butanol fractions of P. crispum subjected to different chromatographic separation techniques. The compounds were identified on the basis of spectral data and compared with previously reported data. Also, the methanolic extract and n-butanol fraction were evaluated for in-vivo hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatotoxicity in mice (10 groups; 8 mice/group). All groups intraperitonially injected by CCl_4 (0.5mg/kg b.w., i.p.) in single dose at 11th day, the treated groups received (100, 200, 300, 400 mg/kg b.w.) of MeOH extract and BuOH fraction separately for 14 days. The results revealed that seven compounds were isolated from EtOAc and n-BuOH fractions which were identified as β-sitosterol (1), quercetin (2), isorhmentin (3), apigenin (4), apigenin-7-O-β-D-glucopyranoside (cosmosiin) (5), diosmetin-7-O-β-D-glucopyranoside (6), apigenin 7-O-β- D- apiofuranosyl (1→2)-β- D glucopyranoside (apiin) (7). On other hand, CCl_4 – induced hepatotoxicity cleared in severe liver damage, showed elevation of liver enzymes. The MeOH extract and n-BuOH fraction exhibited significant hepatoprotection in improvement liver enzymes, with a propensity to return to near-normal levels. So, P. crispum extracts are promising in-vivo hepatoprotective activity.

KEYWORDS: *Apiaceae, β-sitosterol, flavonoid, hepatoprotective activity, Petroselinum crispum.*

INTRODUCTION

The liver is the most essential organ, which organizes several activities, including the metabolism of various nutrients (lipids, carbohydrates, and proteins), and the detoxification of poisons and other foreign materials[1]**.** Many toxins, including carbon tetrachloride (CCl_4), alcohol consumption, thioacetamide, and various chemotherapeutic drugs, cause liver cell injury[1&2]**.** CCl_4 is considered as one of the most potent environmental contaminants. When humans are exposed to CCl_4 via oral, inhalation, and dermal routes, it causes intoxication that associated with high free radical production in several organs, especially the liver and kidney[3]. CCl_4 is widely used to induce hepatotoxicity in experimental animals. The liver is particularly susceptible to oxidative stress due to the direct release of CCl_4 metabolites and cytokines, which reproduce inflammatory responses and their potentially harmful effects. According to reports, this condition resulted in oxidative

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injury, which led to a slew of problems, including liver cirrhosis, cancer, diabetes, atherosclerosis, and other ailments[4]. Synthetically designed drugs used for the prevention and treatment of liver injuries might be insufficient and can cause many side effects. So, many people around the world prefer to use herbal plants for the treatment of diseases. Thus, it is important to find alternative drugs from natural plants that have less toxicity and high efficacy for the treatment of liver disorders[5].

Petroselinum crispum Mill (Parsley) belongs to the Apiaceae family, and widely dispersed over Europe and Asia[6]. *P. crispum* is utilized as a spice, vegetable, and salad element in Egypt. In Arab Traditional Medicine, the leaves of this plant were used to treat inflammation, mastitis, diuretics, laxative, and hematomas[7]. Its roots and seeds have been used to prevent or treat a variety of digestive ailments, including diarrhoea, ulcers, flatulence, and colic pain [7]. It has numerous bioactive ingredients such as flavonoids, phenolic acids, terpenes, organic minerals, thiamin, β-carotene, riboflavin, vitamins C and E[8]. These bioactive constituents have biological features, such as anticancer, antioxidant, insecticidal, antiestrogenic, and antimicrobial activities[9]. The main purposes of the current study were chromatographic isolation and structure elucidation of the nonvolatile constituents of *P. crispum* different extracts using spectroscopic tools including UV, ^{1}H-NMR,^{13}C-NMR, and mass spectra as well as evaluation of their hepatoprotective activities.

MATERIALS AND METHODS

Apparatus and chemicals

Jeol-Delta 2-NMR spectrometer 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}CNMR, Melting point apparatus (SMP 3, Stuart Scientific, UK), UV spectrophotometer spectronic 601 (Milton Roy, USA), and Mass spectra Shimadzu QP-2010 Plus (USA). Authentic samples including Quercetin, Kaempferol, Luteolin, apigenin and Isorhmentin were purchased from Sigma-Aldrich, Germany. Also, all solvents were analytical grade.

Plant Materials

P. crispum aerial parts were collected from Giza governorate, Egypt in March 2018. The plant was identified by Mrs. Teraza Labib a botanist at Orman Garden Herbarium, Giza, Egypt. The voucher specimen (No. C15/3/18) of the plant under investigation has been kept in ideal conditions at the department of Medicinal Chemistry. It was grounded into fine powder for extraction process.

Extraction and Fractionation

3 Kg of dried powder of *P. crispum* aerial parts were extracted using 85% methanol (MeOH) then evaporated under vacuum using a rotatory evaporator (BUCHI, Swetarland). The dried methanol extract (600g) was defatted using pet-ether (PE), the residue was dissolved in 500 ml of water and fractionated by methylene dichloride (CH_2Cl_2), ethyl acetate (EtOAc), and n-butanol (n-BuOH). All fractions were dried well under vacuum using rotatory evaporator and kept in capped vials for chemical and biological experiments.

Experimental animals

Healthy male Swiss albino mice (CD-1 strain), weighting (25 ± 2 g) were purchased from Theodor Bilharz Research Institute (TBRI), Giza, Egypt. The mice were housed in cages for a week to acclimatized at laboratory conditions, (Temp.; 25 ± 2^{o}C, humidity; 55 ± 5% and 12h light/ dark schedules). All animals had free access to water and fed standard pellet diet (24% Protein, 4% fat and 4.5% fiber). All the investigation complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011) and was approved by the Institutional Review Board of TBRI (PT 613; FWA 0010609).

Experimental Protocols

Acute toxicity assessment

Oral acute toxicity studies of MeOH extract and BuOH fraction was carried out according to OECD guidelines-425[10&11]. 36 normal mice were randomly divided into 6 groups (6/group). The first group (control group) received fresh water. The other groups received oral different concentrations of each extract from 100, 500, 1000, 1500 and 2000 mg/kg from each extract separately. Each individual treated mouse was observed (hair erection, walking status, diarrhea, death and body weight) after the first hour then continuously during the following 6 hrs during the first 24 hrs until 14 days.

Carbon tetrachloride (CCl_4) induced hepatotoxicity

10 groups (8 /group) as follows:-

- **Group I (Normal control):** was received distilled water (1 ml/kg, b.w.). **Group II (Toxic control):** was injected CCl4 intraperitonially (i.p.) (0.5 ml/kg b.w. in (1:10) olive oil) for single dose[12].
- **Group III, IV, V, VI (treated groups):** were administrated by oral gavage MeOH extract (100, 200, 300, 400 mg/kg, b.w)[13] once daily for 14 days, respectively.
- **Group VII, VIII, IX, X (treated groups):** were administrated by oral gavage n-BuOH fraction (100, 200, 300, 400 mg/kg, b.w.) once daily for 14 days, respectively. All treated and toxic groups received CCl_4 (0.5 ml/kg, i.p.) once at the11th day.

Blood collection

At the end of treatment, mice were sacrificed under diethyl ether anesthesia. Blood samples were collected and centrifuged. The serum parameters aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) levels were estimated by standard procedures of kits (Biodiagnostic Co. Egypt).

Histopathology studies

Liver was weighed and preserved in 10% neutral buffered formalin. A 5µ thickness of tissue sections was stained with hematoxylin and eosin stain (H and E stain) for histopathological examination.

Preliminary phytochemical screening

Preliminary phytochemical screening of *P. crispum* extracts was conducted to determine the different categories in these extracts, such as sterols, alkaloids, flavonoids, tannins, triterpenoids, saponins, and cardiac glycosides[14, 15].

Isolation and Purification Procedures

The ethyl acetate (EtOAc) and *n*-butanol (n-BuOH) fractions had been subjected to column chromatographic analyses as follows: 15 g of EtOAc was chromatographed to silica gel column chromatography and used gradient solvent of pet-ether / ethyl acetate, ethyl acetate, and ethyl acetate /methanol. The collected fractions had screened by TLC (silica gel, solvent system chloroform: methanol; 8:2). Three main fractions **(A1:A3)** were obtained; **A1** was eluted by (100%ethyl acetate) as white crystals were washed several times by pure methanol to yield compound 1. **A2** was eluted by (95: 5 %; ethyl acetate: methanol) then was re-chromatographed by Sephadex LH-20 column and was purified by preparative paper chromatography using solvent system (acetic acid: water; 20:80) to give compounds 2. **A3 w**as eluted by (90:10%; ethyl acetate: methanol), which was subjected to Sephadex LH-20 and was purified by preparative PC (15% acetic acid) to afford compound 3. On the other hand, 30g of *n*-BuOH fraction was subjected to silica gel column chromatography and was used as gradient solvents pet-ether, pet-ether /ethyl acetate, ethyl acetate, ethyl acetate/ methanol and methanol. Four major fractions were obtained **(B1-B4). B1** was eluted with pure EtOAc, purified using Sephadex LH-20, then using preparative silica gel TLC and (chloroform: methanol; 7:3) as solvent system to give compound 4. **B2** was eluted with chloroform: methanol (95:5) then was re-chromatographed using Sephadex LH-20 column to give compound 5**. B3** was eluted with chloroform: methanol (90:10) was further purified by Sephadex LH-20 column to give compound 6**. B4** was eluted with chloroform: methanol (85:15) was purified by column Sephadex LH-20 and eluted with aqueous methanol several times to afford compound **7**. Four solvent systems were used to follow up the isolation and purification of compounds from fractions which are S1: 15% acetic acid, S2: BAW for PC and S3: (*n*-butanol: methanol: water; 4:1:1), S4: (chloroform: methanol; 8.5:1.5) for TLC. Compounds 2, 3 and 4 were identified by comparison with authentic samples using Co- PC, Co- TLC and HPLC-DAD as quercetin (2), isorhmentin (3) and apigenin (4).

High performance liquid chromatography (HPLC-DAD) profiling of isolated compounds

Ethyl acetate and n-butanol fractions and isolated compounds were dissolved in HPLC grade MeOH and DMSO. Then, they filtered before HPLC analysis. The samples were investigated by Shimadzu LC-8A liquid chromatography system (Shimadzu, Japan) with LC solution software, SPD-M20A photodiode array detector (Shimadzu, Japan). A

RESTEK (USA) C18 column (4.6 mm i.d. × 150 mm) was used and packed with 5 µm particle size. A gradient solvent system with a flow rate of 1 mL min–1 was used for elution which was performed at 25°C. Methanol (B) and water with 1% formic acid (A) were used in the mobile phase. The following was the LC time table starting with 10 % B, 10- 40 %B (5 min), 40- 85 % B (40 min), 85-100 % B (1 min), and 100-100 % B (9 min). The samples (20 µL) were injected for analysis and monitored at different wavelengths for detection of compounds.

Statistical Analysis

The statistical analyses were performed using IBM SPSS (25) software and by one-way analysis of variance (ANOVA) followed by Dunnet.s.t.-test. P values < 0.05 were considered to be significant. The values were expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Acute Toxicity Studies

All doses, (100, 200, 300, 400, up to 2000 mg/kg) of methanol extract and the butanolic fraction were employed for acute oral toxicity studies. It has been found that they are not toxic. The two extracts did not show any hypertension symptoms, diarrhea and /or mortality index in mice, even at the highest dose (2000 mg/kg) [11]. As a result, the examined doses (100, 200, 300, and 400 mg/kg) were safe for further pharmacological research.

Biochemical Estimations

In this study, the toxic effects of CCl_4 caused severe liver damage, which led to higher serum AST, ALT, ALP, and plasma total bilirubin levels in animals that had been treated with CCl_4 than in animals that had not been treated with CCl_4.

Aspartate- aminotransferase (AST) level

Aspartate-Aminotransferase (AST) is divided into two isozymes, one of which is found in the mitochondria and the other in the cytoplasm **.** The results in **Figure 1** were exhibited a highly significant rise in AST level (P <0.01) in the toxic group (96.0±2.6 U/L) when compared with normal group (28.8±3.6 U/L). Mice treated groups with MeOH extract at (100, 200, 300 and 400 mg/kg) reduced significantly the levels of AST (P <0.01) from (77.50±3.8 U/L) to (38.60±2.1 U/L). While, the treated mice groups with different concentrations of *n*-BuOH fraction (100, 200, 300 and 400 mg/kg) led to a significant decrease in AST level (P <0.01) compared with the toxic group. The treatment with *n*-BuOH fraction showed the highest decrease in AST level from (72.4±2.1 to 36.1±2.6 U/L) for serial concentrations (100 to 400 mg/kg). This means that the *n*-BuOH fraction gave higher protection for treated mice than the MeOH extract. **Pendit** *et al*.,[16] reported that the treatment of CCl_4-administrated rats with a dose of 200 mg/kg b.w. of *Aghatoda vasica* leaves extract led to a decrease in the elevated AST level compared with the toxic group. Also, **Maqsood** *et al*.,[17] reported that the pre-treatment of CCl_4-treated mice with extracts of *Polygonum amplexicaule* at a dose of 200 mg/kg resulted in a significant reduction of AST levels from 62 to 94.4%. Also, **ERTAŞ** *et al*.,[18] proved the improvement effect of *P. crispum* extract on AST and ALT levels.

Alanine-aminotransferase (ALT) level

Alanine-aminotransferase (ALT) is found in the plasma membrane of hepatocytes, and an increase in ALT levels is linked to damage of hepatocytes' liver membrane[18]**.** An elevated level of ALT in the toxic group compared with the normal group and also, the decreasing levels of ALT of treatment mice with various concentrations of the MeOH extract and *n*-BuOH fraction (100, 200, 300, and 400 mg/kg) compared with the toxic group were showed at **Figure 2.** The results showed that toxic group exhibited a very highly significant increase (P<0.01) in ALP level (92.0±1.9 U/L) as compared to normal control (17.0±2.1 U/L). The higher ALT parameter of the serum indicated the toxic potential of CCl_4, which causes more damage to the liver. The treatment of mice with multiple concentrations of the MeOH extract (100, 200, 300, and 400 mg/kg) led to a significant reduction (P< 0.01) of the ALT level from (87.3 ± 7.5 to 43.0 ± 1.7U/L). While the groups treated with *n*-BuOH fraction demonstrated a higher significant decrease (P< 0.01) of ALT level from (82.4 ± 2.1 to 41.5 ± 4.7 U/L) when compared with the toxic group, as shown in **Figure 2** exhibited the treatment of mice with a high concentration (400 mg/kg) of each extract showed the highest reduction in ALT levels. These findings are in agreement with reported studies[19-21].

Alkaline phosphatase (ALP) level

The most reliable enzyme for detecting hepatic injury to hepatocytes is alkaline phosphatase (ALP), which is a liver parenchymal more selective protein [20]. The results in **Figure 3** revealed that the toxic group showed a highly significant increase ($P< 0.01$) in ALP level (179.3 ± 9.4 U/L) as compared with the normal group (110.8 ± 9.6 U/L). This increase refers to the damage of the liver due to its localization in cytoplasm and released after cellular damage[16, 20]. The results exhibited the effect of the MeOH extract to a highly significant decrease ($P< 0.01$) of ALP levels ranging from (160.9 ± 5.2 U/L) for MeOH (100 mg/Kg) to (131.4 ± 6.2 U/L) for MeOH (4 00 mg/Kg). In addition, the *n*-BuOH fraction gave a highly significant decrease ($P< 0.01$) of ALP levels from (150.3 ± 6.8 U/L) to (124.8 ± 4.8 U/L) for *n*-BuOH (100 mg/Kg to 400 mg/Kg), respectively. It has appeared that both extracts showed a decrease in the ALT levels of the toxic group, and the *n*-BuOH fraction gave a higher lowering of the ALT levels than the MeOH extract. These results were in line with several reports exhibited that *P.crispum* extract decreases levels of all liver enzymes (ALT, AST, ALP)[21&22]

Total bilirubin (TB) level

Estimation of Plasma Bilirubin Level is also the most essential assay used to diagnose hepatic disorders. It assesses the binding, excreting capability and conjugation of hepatocytes proportionate to the rate of erythrocyte deterioration [20]. The results in **Figure 4** showed a highly significant increase of the total bilirubin level ($P< 0.01$) in the toxic group (1.2 ± 0.10 U/L) as compared to the normal group (0.4 ± 0.05 U/L). This rise in serum bilirubin reflects the excess of bilirubin production due to RBCs (red blood cells) breakdown as result of liver injury[16]. While the other groups treated with different concentrations (100-400 mg/Kg) of the MeOH extract exhibited a high significant reduction ($P< 0.01$) in TB levels from (1.1 ± 0.05 U/L) to (0.6 ± 0.04 U/L). On the other hand, the *n*-BuOH fraction possesses a highly significantly lowering ($P<0.01$) in TB levels from (1.0 ± 0.03 U/L) to (0.57 ± 0.03 U/L) for *n*-BuOH (100 to 400 mg/Kg), respectively. These results revealed that the MeOH extract and *n*-BuOH fraction had a protective effect against CCl_4-administered mice. Also, the *n*-BuOH fraction exhibited much higher protection than the MeOH extract. The previous results exhibited a significant decrease ($P< 0.01$) in hepatic enzymes (AST, ALT, ALP and TB) of *n*-BuOH fraction and the MeOH extract-treated groups when compared to CCl_4 administrated mice group.

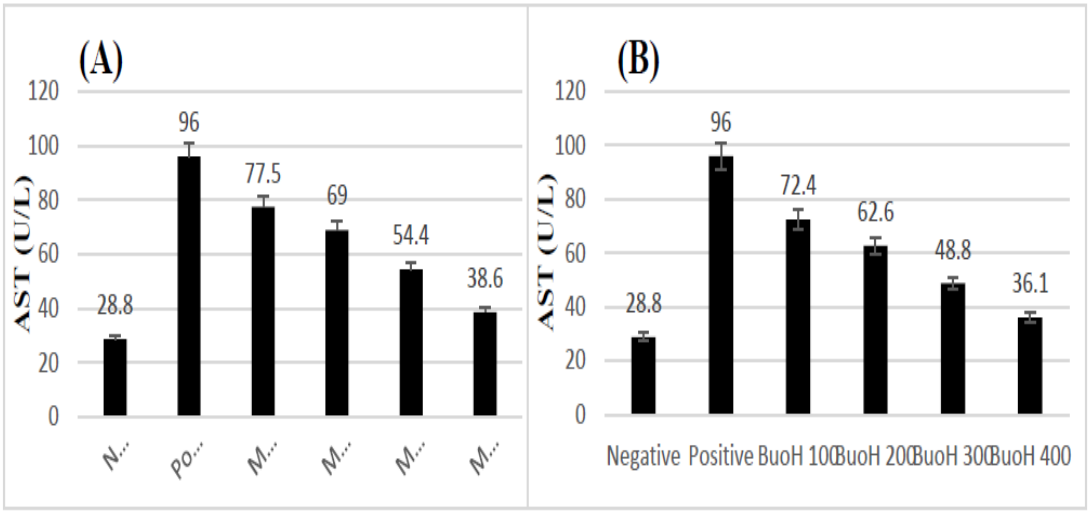

Fig. 1: Effect of *P. crispum (A)* 85% MeOH extract, (B) n-BuOH fraction on induced hepatotoxicity-related AST parameter.

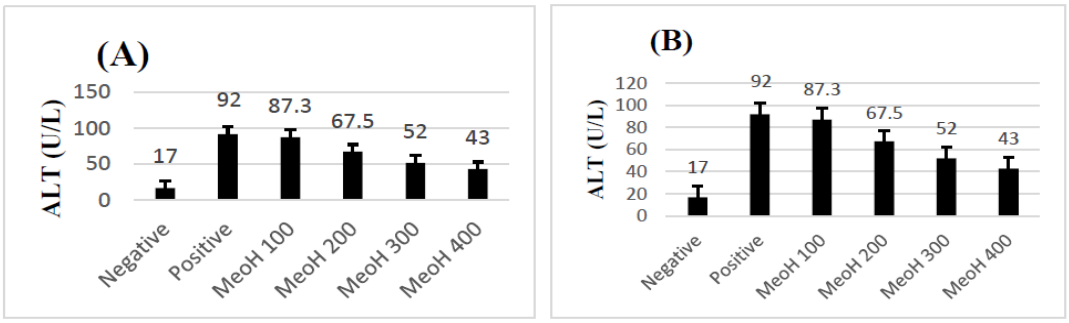

Fig. 2: Effect of *P. crispum (A)* 85% MeOH extract, (B) n-BuOH fraction on induced hepatotoxicity-related ALT parameter

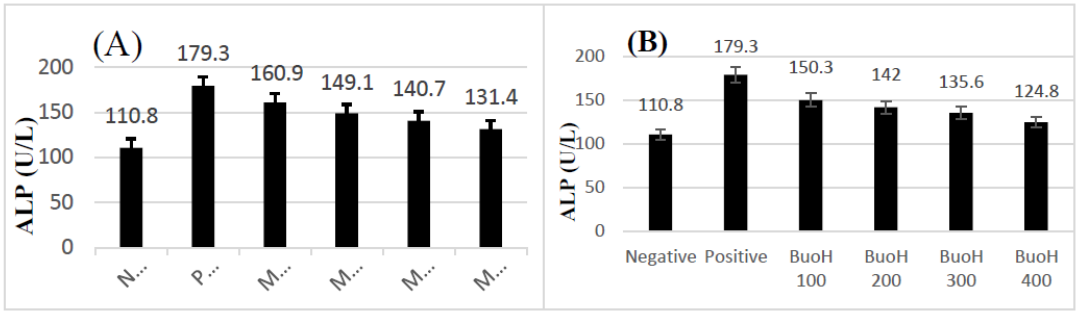

Fig. 3: Effect of *P. crispum (A)* 85% MeOH extract, (B) n-BuOH fraction on induced hepatotoxicity-related ALP parameter.

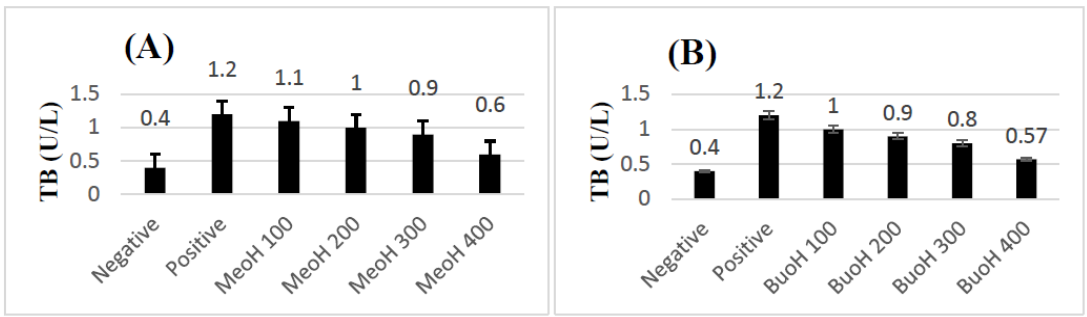

Fig. 4: Effect of *P. crispum (A)* 85% MeOH extract, (B) n-BuOH fraction on induced hepatotoxicity-related TB parameter**.**

Histopathological Changes

The results of histopathological studies (**Figure 5**) are in full agreement with biochemical analysis. The normal animals' liver sections showed typical cellular architecture, including real cytoplasm, a conspicuous nucleus, a defined central vein, and hepatic lobules that were free of fatty alterations or necrosis as shown in **Figure 5-A**. Hepatic cells with serious toxicity were identified by histology of CCl_4-treated mice's liver sections, which showed extensive loss of hepatic architecture with intense peripheral and central vein necrosis, apoptosis, cytoplasm vacuolization, centrilobular fatty degeneration, and congestion of hepatocyte sinusoids (**Figure 5-B**). A hepatocyte swelling, hepatocyte atrophy, hepatocyte degeneration, proliferation of Kupffer cells, and minor fatty alterations with central vein injury were observed after treatment with a low dose of plant extracts (100- 400 mg/Kg). On contrary, the high doses of the same extracts revealed typical hepatic architecture with only a minor accumulation of fatty lobules as shown in **Figure 5 (C-J).**

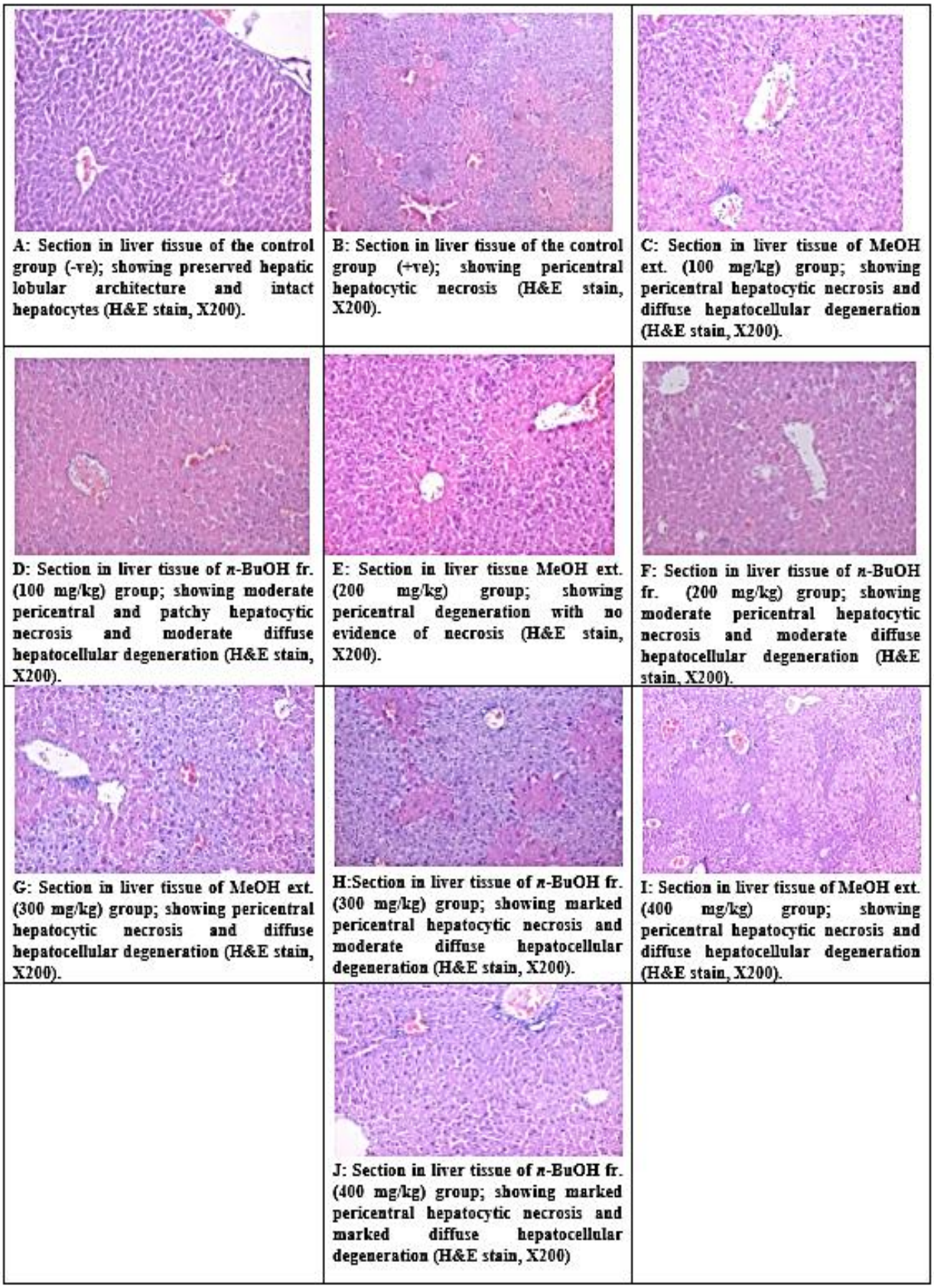

Fig. 5: The histopathological changes showing effect of the tested extracts (A-J) on the mice induced by CCl_4.

Preliminary phytochemical screening

In the present study, the preliminary phytochemical analysis of *P. crispum* extracts illustrated that the EtOAc and n-BuOH fractions have a high amount of phenols and flavonoids. While, they have a moderate amount of tannins, triterpenes, saponins, cardiac glycosides and a low amount of alkaloids. Whereas, the 85% MeOH extract

contains all these secondary metabolites ranging from moderate to low concentrations. In addition, the residue had a low amount of flavonoids, cardiac glycosides and phenols as well as other phytochemicals are absent as shown in **table (1)**. These findings are generally agreeing with previous studies carried out on different parts of *P. crispum*[7].

Phytochemical analysis

Seven compounds were isolated as one sterol and six flavonoids **Figure (6)** . β-sitosterol was the first time to be isolated from *P. crispum.* The compounds were identified by different spectroscopic analyses and (HPLC-DAD) chromatography as shown in **Figure (7 and 8).** The high hepatoprotective activity of the extracts is due to the number of flavonoids that are polyphenol compounds that have been proven for hepatocytes protection[23]

Table 1: Preliminary phytochemical screening of *P. crispum* aerial parts extracts.

Phytochemicals	85% MeOH ext.	CH_2Cl_2 fr.	EtOAc fr.	*n*-BuOH fr.	Residue
Flavonoids	++	+	+++	+++	+
Alkaloids	+	-	+	+	-
Tannins	+	+	++	++	-
Sterols	+	++	++	++	-
Triterpenoids	+	+	++	++	-
Cardiac glycosides	++	+	++	++	+
Phenols	++	++	+++	+++	+
Saponins	+	-	+	++	-

(+++): high amount, (++): moderate amount, (+): low amount, (-): Absent.

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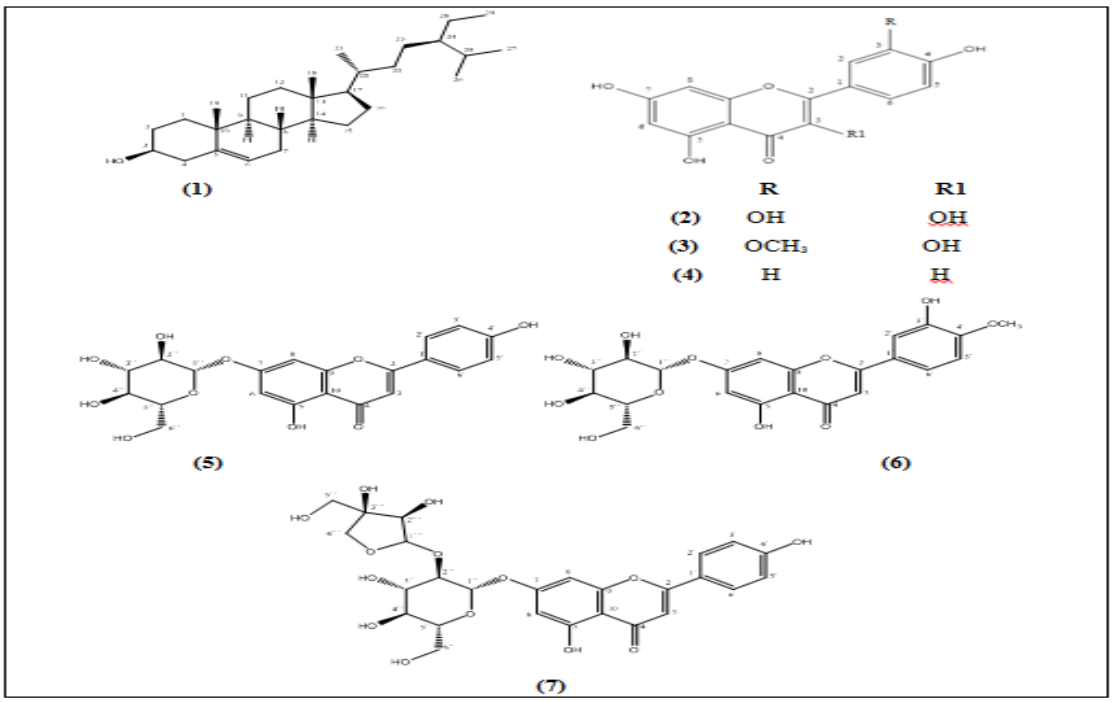

Fig. 6: Structure of the isolated compounds**.**

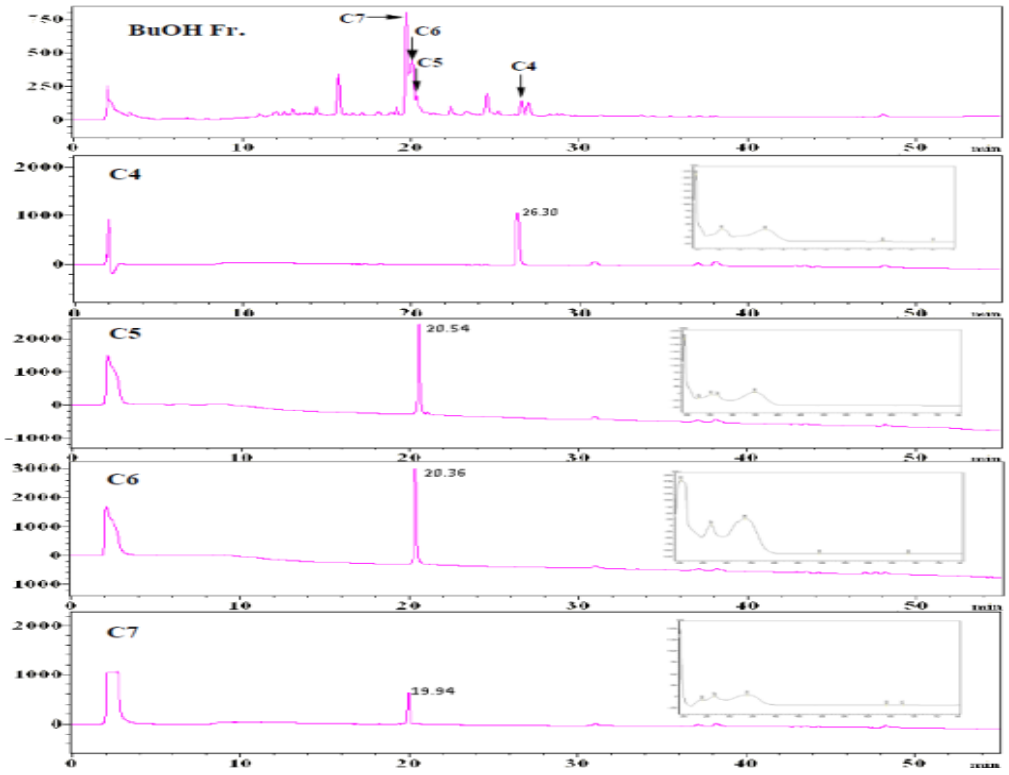

Fig. 7: HPLC-DAD profile of EtOAc fraction and its pure isolated compounds (1-3).

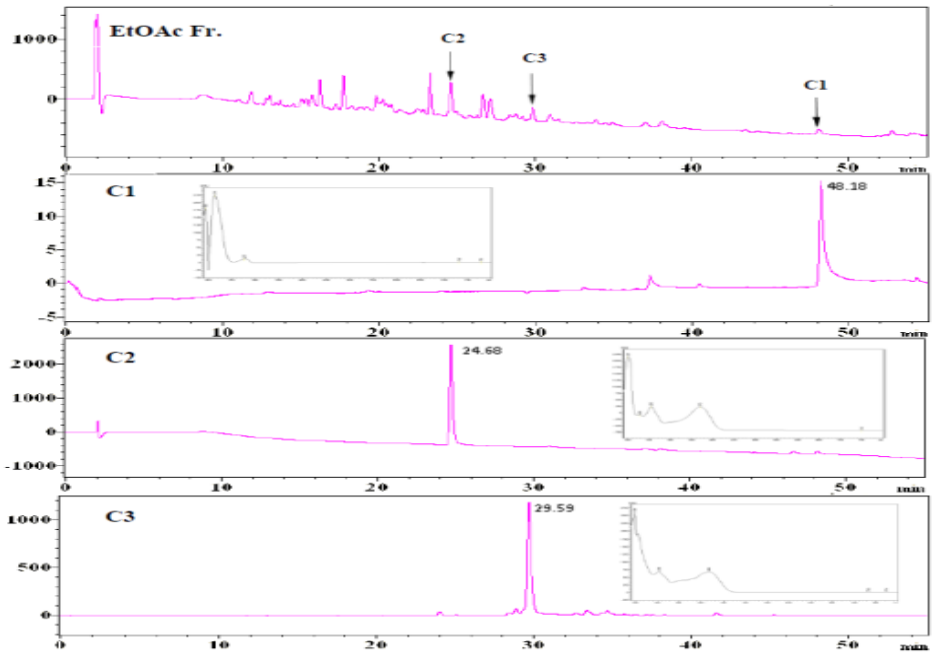

Fig. 8: HPLC-DAD profile of n-BuOH fraction and its pure isolated compounds (4-7)**.**

Identification of isolated compounds

β-sitosterol (1)

amorphous white crystals. m.p. 148-150°C. HPLC-DAD: (t_R) = 48.18 min; UV λ_{max} $_{(nm)}$ (MeOH): 277. ESI-MS m/z: 414, 437 [M^++Na], 301 [M^+-113] or loss of (-C_8H_{17}). ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.44 (2H, m, H-1), 1.56 (2H, m, H-2), 3.3 (1H, m, H-3), 2.16 (2H, m, H-4), 5.27 (1H, br s, H-6), 2 (2H, m, H-7), 1.68 (1H, m, H-8), 1.54 (1H, m, H-9), 1.52 (2H, m, H-11), 1.5 (2H, m,H-12), 1.49 (1H, m,H-14), 1.58 (2H, m, H-15), 1.83 (2H, m, H-16), 1.42 (1H, m, H-17), 0.67 (1H, s, H-18), 0.97 (1H, s, H-19), 1.58 (2H, m, H-20), 0.93

(3H,d, H-21), 0.92 (2H, m, H-22), 1.12 (2H,m, H-23), 1.37 (1H,m, H-24), 1.57 (1H, m, H-25), 0.83 (3H, d, J=6.2, H-26), 0.85 (3H, d, J=6.2, H-27), 1.01 (2H, m, H-29), 0.81 (3H, t, H-29). ^{13}C-NMR (DMSO-d_6) δ (ppm): 37.4 (C-1), 32.01(C-2), 70.57 (C-3), 42.74 (C-4), 141.7 (C-5), 120.9 (C-6), 31.93 (C-7), 31.93 (C-8), 50.18 (C-9), 36.61 (C-10), 21.15 (C-11), 39.8 (C-12), 42.39 (C-13), 56.77 (C-14), 24.41(C-15), 28.32 (C-16), 55.98 (C-17), 12.3 (C- 18), 19.65 (C-19), 36.05 (C-20), 19.1 (C-21), 33.92 (C-22), 26.01 (C-23), 45.73 (C-24), 29.23 (C-25), 20.2 (C-26), 19.41 (C-27), 23.14 (C-28) and 12.17 (C- 29).

Quercetin (2)

yellow powder. m.p. 314-316˚C. R_f: 0.08 (S1), 0.66 (S2). HPLC-DAD: (t_R) = 24.68 min; UV λ_{max} (nm) (MeOH): 255, 370; + MeOH / NaOMe 270, 413; + MeOH /$AlCl_3$ 275, 455; +MeOH /$AlCl_3$+HCl 268, 425; + MeOH / NaOAc 274, 389; + MeOH / NaOAc + H_3BO_3 258, 385.

Isorhmentin (3)

yellowish brown powder. m.p. 310-312 ˚C. R_f: 0.10 (S1), 0.56 (S2). HPLC-DAD: (t_R) = 29.59 min; UV λ_{max} (nm) (MeOH): 254, 300^{sh}, 365. ESI-MS m/z: 315 $[M-H]^-$. ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.23 (1H, d, J= 2.1, H-6), 6.51 (1H, d, J= 2, H-8), 7.79 (1H, d, J=6.1, H-2′), 6.97 (1H, d, J = 2.1, H-5′), 7.72 (1H, m, H-6′), 3.88 (3H, s, OCH3).

Apigenin (4)

pale yellow powder. m.p. 351-353˚C, R_f: 0.1 (S1), 0.62 (S2), 0.38 (S3). HPLC-DAD: (t_R) = 26.3 min; UV λ_{max} (nm) (MeOH): 256, 358; + MeOH / NaOMe 270, 412; + MeOH /$AlCl_3$ 272, 422; +MeOH /$AlCl_3$+HCl 271, 411; + MeOH / NaOAc 273, 401; + MeOH / NaOAc + H_3BO_3 267, 382.

Apigenin-7-O-β-D-glucopyranoside (cosmosiin) (5)

yellowish amorphous powder. m.p. 201-203˚C. R_f: 0.13 (S1), 0.38 (S4). HPLC-DAD: (t_R) = 20.54 min; UV λ_{max} (nm) (MeOH): 253 / 267^{sh}, 349 nm; ESI-MS m/z: 431 $[M-H]^-$.^{1}H-NMR (DMSO-d_6) δ (ppm): 6.87 (1H, s, H-3), 6.48 (1H, s, H-6), 6.88 (1H, s, H-8), 6.98 (2H, d, J= 7.8, H-3′, H-5′), 7.99 (2H, d, J= 7.8, H2′, H-6′), 5.47 (1H, s, H-1″), sugar protons (3.73-4.68). ^{13}C-NMR (DMSO-d6) δ (ppm): 164.84 (C-2), 103.63 (C-3), 178.04 (C-4), 162.07 (C-5), 98.55 (C-6), 163.54 (C-7), 95.40 (C-8), 157.52 (C-9), 105.91 (C-10), 121.51 (C-1'), 129.15 (C-2′ and C-6′), 116.58 (C-3′ and C-5′), 159.40 (C-4′), 100.45 (C–1″), 73.65 (C–2″), 76.98 (C–3″), 70.09 (C–4″), 77.72 (C–5″) and 61.94 (C–6″).

Diosmetin-7-O-β-D-glucopyranoside (6)

yellowish amorphous powder, m.p. 252-254˚C. R_f: 0.20 (S1), 0.34 (S4). HPLC-DAD: (t_R) = 20.36 min; UV λ_{max} (nm) (MeOH): 267, 336; ESI-MS m/z: 461 $[M-H]^-$, ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.90 (2H, br s, H-8, H-3), 6.47 (1H, br s, H-6), (1H, br s, H2′), 6.99 (1H, br s, H-5′),7.61 (1H, br s, H-6′), 5.09 (1H, br s, H-1″), sugar protons (3.50-4.70), 3.92 (3H, s, OCH3). ^{13}C-NMR (DMSO-d_6) δ (ppm): 164.56 (C-2), 103.79 (C-3), 182.14 (C-4), 163.67 (C-5), 100.04 (C-6), 164.56 (C-7), 95.25 (C-8), 156.95 (C-9), 105.77 (C-10), 121.13 (C-1′), 114.62 (C-2′),146.12 (C-3′), 151.48 (C-4′),116.39 (C-5′), 119.66 (C-6′), 100.36 (C-1″), 73.75 (C-2″),76.95 (C-3″), 70.56 (C-4″), 77.81 (C-5″), 61.22 (C-6″) and 50.27 (OCH3).

Apigenin 7-O-β-D- apiofuranosyl (1→2)-β-D glucopyranoside (apiin) (7)

yellowish amorphous powder, m.p. 229-231˚C. R_f: 0.36 (S1), 0.46 (S4). HPLC-DAD: (t_R) = 19.94 min; UV λ_{max} (nm) (MeOH): 267, 338; ESI-MS m/z: 563 $[M–H]^-$. ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.84 (1H, s, H-3), 6.43 (1H, br s, H-6), 6.85 (1H,br s, H-8), 7.99 (1H, d, H-2′, H-6′), 6.98 (1H, d, H-3′, H-5′), 5.21 (1H, br s, H-1"), 5.39 (1H, br s, H-1'"). ^{13}C-NMR (DMSO-d_6) δ (ppm): 164.24 (C-2), 103.08 (C-3), 181.92 (C-4), 161.39 (C-5), 99.42 (C-6), 162.78 (C-7), 94.80 (C-8), 156.84 (C-9), 105.45 (C-10), 121.01 (C-1′), 128.48 (C-2′, C-6′), 115.93 (C-3′, C-5′), 161.08 (C-4′), 98.26 (C-1"), 75.98 (C-2"), 76.27 (C-3"), 69.85 (C-4"), 77.00 (C-5"), 60.64 (C-6"), 108.77 (C-1'"), 76.85 (C-2'"), 79.16 (C-3'"), 73.82 (C-4'") and 64.25 (C-5'").

Conclusion

The pre-treatment of mice with the 85% MeOH extract and *n*-BuOH fraction of *P. crispum* exhibited an improvement in the enzymes of the liver, with a propensity to return to near-normal levels against CCl_4 induced hepatotoxicity. This reflects that the 85% MeOH extract and *n*-BuOH fraction were promising hepatoprotective agents and the histopathological changes of the examined liver

sections have been supported these findings. Therefore, the use of *P. crispum* as a spice in our diet or as a supplement on a regular basis can aid the body's antioxidant systems in reducing the detrimental effects of oxidative stress and the problems of liver diseases.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation**.**

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نشرة العلوم الصيدلية
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فصل كروماتوجرافي و تعريف وتوصيف لبعض المركبات الكيميائيه لاوراق نبات بيتروسلينيم كرسبم و تقييمها كعوامل واقيه للكبد

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نبات بتروسلينوم كريسبوم الذى ينمو فى مصر و الذى يستخدم على نطاق واسع كتوابل أساسية بسبب فوائده الصحية. تهدف هذه الدراسة إلى التعرف على مكوناته الكيمائيه و تقييم مستخلصاته كواقيات للكبد. وذلك بالتعرف على المحتوى الكيميائى للمستخلصات المختلفه باستخدام جهاز كروماتوجرافيا السائل عالي الكفاءة المتصل بكاشف الاشعة الفوق بنفسجية (HPLC-DAD). كما تم اجراء فصل كروماتوجرافي للمستخلصات الاعلي كفاءة باستخدام طرق الفصل الكروماتوجرافي المختلفة، وتم تعريف التركيب الكيميائي الدقيق للمركبات النقية المفصولة باستخدام الطرق الطيفية المختلفة مثل الأشعة فوق البنفسجية وطيف الكتلة والرنين النووي المغناطيسي.

وكانت نتائج الفصل الكروماتوجرافي لمستخلص خلات الإيثيل عباره عن ثلاث مركبات تم تحديد التركيب الكيميائي الدقيق وتعريفهم على النحو التالي :

β-sitosterol (١), quercetin (٢) and isorhmentin (٣).

كما أدى الفصل الكروماتوجرافي لمستخلص البيوتانول إلى فصل وتحديد التركيب الكيميائي الدقيق لأربعة مركبات تم تعريفها على النحو التالي :

Apigenin (٤), apigenin-٧-O-β-D-glucopyranoside (٥), diosmetin-٧-O-β-D-glucopyranoside (٦), and ٧-O-β-D- apiofuranosyl (١→٢)-β-D-glucopyranoside (٧).

تم تقييم تاثير كلا من المستخلص الميثانولي و البيوتانولى على النشاط الكبدي داخل الجسم الفئران ضد السمية الكبدية التي يسببه مركب رابع كلوريد الكربون بجرعات مختلفه. اظهر نتائج كلا من المستخلص الميثانولي و البيوتانولى تحسنًا في إنزيمات الكبد ، مع ميل للعودة إلى المستويات شبه الطبيعية ضد السمية الكبدية التي يسببها رابع كلوريد الكربون. لذلك تعتبر مستخلصات بتروسلينوم كريسبوم لها نشاطًا واعدًا في حماية الكبد في الجسم الحي.