

Flavones composition and therapeutic potential of *Dodonaea viscosa* against liver fibrosis

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Abstract

Dodonaea viscosa (L.) Jacq (Sapindaceae) is used in traditional medicine for the treatment of hemorrhoids, ulcer, and pains of hepatic or stomach origin. The current study was designed to investigate the phytochemical constituents of the plant and evaluate its activity against liver fibrosis induced by carbon tetrachloride (CCl₄) in rats. The phytochemical analysis has afforded one flavanone; 5,7-dihydroxy flavanone (pinocembrin) (1) and eight flavones. The compounds were isolated and elucidated as; 5,7-dihydroxy-3,6,4'-trimethoxyflavone (santin) (2), 5,7,4'-trihydroxy-3-methoxy flavone (kaempferol 3-O-methyl ether) (3), 3,4',5-trihydroxy-7-methoxy flavone (kaempferol 7-O-methyl ether) (4), 3',4',5,7-tetrahydroxy-3-methoxy-flavone (quercetin 3'-O-methyl ether) (5), 3,3',4',5,7-pentahydroxyflavone (quercetin) (6), 5,7,4'-trihydroxy-3,6-dimethoxy flavone (7), 5,7-dihydroxy 3,6,3',4'-tetramethoxy flavone (8), and isorhamnetin-3-O-robinobioside (9).. In vitro screening of ethanol extract, fractions of toluene and ethyl acetate, the flavanone and major flavone compounds as antioxidants was carried out. In addition, *D. viscosa* ethanol extract and two fractions were examined in vivo against liver fibrosis induced by CCl₄ in rats. The evaluation was done through measuring hepatic oxidative stress markers; malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD). The work was extended to measure serum protein content and liver function enzymes; aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT). Liver histopathological analysis was done for results confirmation. In conclusion, among the isolated flavones, compounds 3, 4, and 8 were isolated for the first time. The ethanol extract and compound of 6, 5 followed by 7 exhibited the strongest antioxidant activities. Treatment with *D. viscosa* extract and its fractions attenuates the increments of MDA, AST, ALT, ALP, GGT, total protein and increased GSH and SOD levels. The ethanol extract showed the most potent effect. The data confirmed the rationale for the traditional use of *D. viscosa* extracts to hepatic disorders. Further studies are needed in order to evaluate the isolated flavones as protective agents against liver injury and for their clinical application.

Keywords: *Dodonaea viscosa*, flavones, phytochemical constituents, liver fibrosis, antioxidants, histopathology.

Introduction

The genus *Dodonaea* (Sapindaceae) is comprised of approximately 68 species. Previous reports have demonstrated that some traditional medicinal plants from the genus *Dodonaea* are rich in flavonoid components [1-4]. *Dodonaea viscosa* (L.) Jacq. is an evergreen shrub widely found in tropical and subtropical regions, and it has been used in traditional medicines in various countries [5, 6]. In various African countries, Surah region of Saudi Arabia and other Asian countries, people administered orally the dried leaves decoction for treatment of stomach ulcer after grinding and mixing with milk or honey, hemorrhoids, and stomachache pains of hepatic or splenic origin [3, 4, 6]. Besides the traditional uses, some pharmacological studies give an insight of its therapeutic potentials as anti-

inflammatory, antioxidant and hypolipidaemic effect were reported [7-9].

Liver, an imperative organ, has a crucial role in the metabolic pathways that causes liver to succumb to numerous hepatic diseases. Carbon tetrachloride (CCl₄), as a xenobiotic, caused oxidative stress and may injures hepatic cells [10]. Many studies have established the fact that CCl₄ is metabolized in the liver into a highly reactive substance, trichloromethyl, which initiate free radicals that mediate lipid peroxidation process [11].

Synthetic drugs exploited in the treatment of liver diseases are incompetent and may sometimes lead to serious side effects [12]. In this regard, herbal therapy has emerged as proficient approach with good values in treating hepatic diseases.



Plants offer a wide range of natural antioxidants due to the structural diversities of their secondary metabolites; mainly phenolic compounds, with higher potency and lower toxicities than the synthetic ones currently available [13, 14]. They may also serve as vital source of potentially useful new agents for development of effective therapy to combat a variety of liver problems with interception of fewer side effects [12, 13, 15].

Despite the favorable ethnopharmacological properties of *D. viscosa*, its protective effect against liver fibrosis has not previously been explored. The aim of the present study was to evaluate the ethanolic extract of *D. viscosa* and the fractions of toluene and ethyl acetate against liver fibrosis induced by CCl_4 in rats. The evaluation was done through measuring certain oxidative stress markers, liver function indices and histological analysis of the liver sections.

Materials and methods

Chemicals

All chemicals in the present study were high analytical grade products from Sigma (USA), Merck (Germany), BDH (England), Riedel de H en (Germany) and Fluka (Switzerland).

Plant materials

The aerial parts of *Dodonaea viscosa* L. were collected from mountains of Al-Aqiq Valley in Al-Baha, Saudi Arabia at altitude of 800-1200m height in May 2008. They were identified by Dr. Farag Abd-Allah Alghamdi, Botany Department, King Abdul-Aziz University, Jeddah, Saudi Arabia. Voucher specimens (number: N012456) were deposited in King Abdul-Aziz University Herbarium.

Material for chromatography

Precoated silica gel 60 F_{254} plates for thin layer chromatography (TLC) and Whatman No. 1 sheets (Whatman Ltd., Maidstone, Kent, England) for paper chromatography (PC) were used. Column chromatography (CC) was carried out using silica gel (Si) 60 mesh of 35-60 and 60-120 (E. Merck, Darmstadt, Germany) or Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

Apparatus

Electrothermal digital and Gallenkamp electrothermal melting point Apparatus were used. Spectrophotometer model UV – 240 (200-600 nm). The NMR spectra were recorded at 300 (^1H) and 75 (^{13}C) MHz on Varian Mercury 300 (Varian, UK) NMR spectrometer; values are reported as ppm relative to TMS in the convenient solvent. EI-MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) (Finnigan MAT, San Jose, CA). HRESI/MS was carried out using Bruker microTOF spectrometer (Bruker-

Daltonics, CA). UV-VIS spectra were recorded on a Perkin-Elmer UV/VIS spectrometer (Perkin-Elmer, Norwalk, CT, USA).

Solvent systems and spray reagent

S_1 : Benzene/EtOAc (7:3); S_2 : Benzene/EtOAc (6:4); S_3 : $\text{CHCl}_3/\text{MeOH}$ (9:1) were used for TLC, while S_4 : n-BuOH/HOAc/ H_2O (4:1:5, top layer); S_5 : 15 % aqueous HOAc were for the PC. All solvents used for extractions and chromatographic separations were of analytical grade. AlCl_3 spray reagent (1 g powder of AlCl_3 in 100 mL of ethanol) was used. After spraying, heat the dry chromatogram at 105 $^\circ\text{C}$ for 10 min. and visualize under UV light.

Phytochemical study

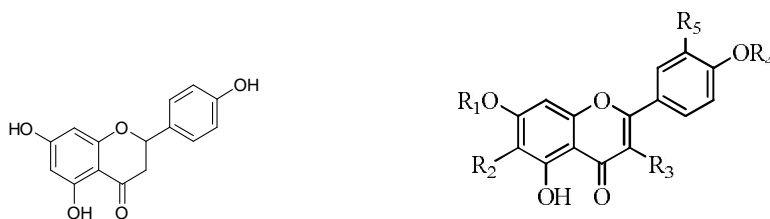
Extraction, fractionation and isolation

The air dried and powdered aerial parts of *D. viscosa* (2.5 kg) were extracted with 80% ethanol at room temperature. The extract was concentrated under reduced pressure and temperature not exceeding 50 $^\circ\text{C}$ to yield 308.9 g (12.36 % dry weight basis). An aliquot of the ethanolic extract (150 g) was suspended in water and fractionated by extraction with toluene and ethyl acetate till exhaustion. The solvent-free extractives (41.28 and 26.48 g, respectively) were saved for isolation of the major constituents. The fractions were screened by TLC and/or PC using S_1 – S_5 as solvent systems. The chromatograms were examined under UV light before and after exposure to ammonia vapor, as well as, after spraying with the reagent. Part of toluene fraction (30 g) was fractionated on a Si CC. Elution was carried out using petroleum ether, petroleum ether-toluene mixtures with increasing polarity till toluene, then ethyl acetate. Fractions, 100 mL each, were collected and monitored by TLC, using S_1 – S_3 as solvent systems and AlCl_3 spray reagent. Fractions of similar chromatographic profile were pooled to yield four main fractions. These fractions were chromatographed on different columns viz., silica gel column chromatography (eluted successively with a step gradient of toluene/EtOAc (100:0 - 0:100 v/v) then gradual increase polarity with methanol) and Sephadex LH-20 using methanol as eluent. Compounds 1 – 6 were isolated, their purity being checked by comparative TLC using solvent systems S_1 – S_3 . The ethyl acetate fraction was subjected to Si CC; being eluted with n-hexane-ethyl acetate to give three fractions. The fractions were monitored by TLC and/or PC, using S_3 – S_5 for the isolated compounds (7 – 9).

Identification of compounds

The structures of isolated compounds were elucidated by spectroscopic methods including UV, NMR, EI-MS, and HR-EMS as well as comparison with published data [16 - 19].





	R ₁	R ₂	R ₃	R ₄	R ₅
5,7-dihydroxy flavanone (pinocebrin) (1)					
5,7-Dihydroxy-3,6,4'-trimethoxyflavone (Santin) (2)	H	OCH ₃	OCH ₃	CH ₃	H
5,7,4'-Trihydroxy-3-methoxy flavone (Kaempferol 3-O-methyl ether) (3)	H	H	OCH ₃	H	H
3,5,4'-Trihydroxy-7-methoxy flavone (Kaempferol 7-O-methyl ether) (4)	CH ₃	H	OH	H	H
3,5,7,4'-Tetrahydroxy-3-methoxy flavone (Quercetin 3-O-methyl ether) (5)	H	H	OH	H	OCH ₃
3,5,7,3,4-Pentahydroxyflavone (Quercetin) (6)	H	H	OH	H	OH
5,7,4'-Trihydroxy-3,6-dimethoxy flavone (7)	H	OCH ₃	OCH ₃	H	H
5,7-Dihydroxy-3,6,3',4'-tetramethoxy flavones (8)	H	OCH ₃	OCH ₃	CH ₃	OCH ₃
Isorhamnetin-3-O-robinobioside (9)	H	H	Gal-Rha	H	OCH ₃

Fig. 1: Chemical structures of *Dodonaea viscosa* isolated compounds.

Bioactivity assays

In vitro antioxidant effect

In vitro antioxidants screening of *D. viscosa* ethanol extract, its toluene and ethyl acetate fraction as well as the flavanone (1) and major flavones (2, 5-9) was carried out by the method of Chen et al. (2007) [20]. Serial concentrations (10:100 μ g) for each tested sample were estimated, where 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals react with plant antioxidants and the decrease in absorbance (A) of DPPH was calculated in relation to absorbance of control as follows:

$$\% \text{ IP} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

In vivo study

Animals

Male Wistar albino rats (100: 120 g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet. Anesthetic procedures and handling with animals complied with the ethical

guidelines of Medical Ethical Committee of National Research Centre in Egypt.

Doses and root of administration

Administration regimen was twice a week for six consecutive weeks. CCl₄ (0.5 mL/kg) was suspended in olive oil (1:9 v/v) and injected intraperitoneally [21]. Ethanolic extract and the fractions of toluene and ethyl acetate of *Dodonaea viscosa* were, separately, administrated orally at a dose of 200 mg/kg [8]. Silymarin; a reference herbal drug was orally administered at a dose of 100 mg/kg [22].

Experimental groups

48 Male Wistar strain albino rats were used in this study. Animals were divided into 6 groups (8 rats each). Group 1 served as normal healthy control rats. Group 2 injected with CCl₄. Groups 3 - 5 forced at the same time with CCl₄ and *D. viscosa* extracts (ethanol, toluene and ethyl acetate). Group 6 forced with CCl₄ and silymarin drug.

Biological sample preparations

Liver tissue was homogenized in normal physiological saline solution (0.9% NaCl) (1:10 w/v). The homogenate was centrifuged at 3000 xg for 5 minutes (4 °C) and the supernatant was used for estimation of hepatic oxidative stress markers.

Serum sample: Blood collected from each animal by puncture the sublingual vein in clean and dry test tube, left 10 min to clot and centrifuged at 3000 xg (4°C) for serum separation. The separated serum was stored at -80 °C for further determinations of liver function enzymes and serum protein.

Biochemical assays

Oxidative stress markers

Malondialdehyde; a marker of lipid peroxidation process was estimated by method of Buege and Aust (1978) at 535 nm [23]. Glutathione was assayed by Moron et al. (1979) using dithiobis-2-nitrobenzoic acid (DTNB) in PBS at 412 nm [24]. Superoxide dismutase was estimated by method of Nishikimi et al. (1972) [25], where the increase in absorbance of reduced nicotinamide adenine dinucleotide (NADH) was measured at 560 nm using its molar extinction coefficient $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Serum biomarkers for liver function tests

Aspartate and alanine aminotransferases were measured by the method of Gella et al. (1985) [26], where the transfer of amino group from aspartate or alanine formed oxalacetate or pyruvate, respectively and the developed colour was measured at 520 nm. Alkaline phosphatase catalyzed in alkaline medium the transfer of phosphate group from 4-nitrophenol to 2-amino-2-methyl-1-propanol (AMP) and liberated 4-nitrophenol. The developed colour was measured at 510 nm [27]. GGT was estimated by the method of Szasz (1969) [28], where GGT enzyme reacted with L-glutamyl-3-carboxy-p-nitroanilide and glycyl-glycine to give L-glutamyl-glycyl-glycine and 5-amino-2-nitrobenzoate. The decrease in absorbance was read at λ_{max} of 450 nm at 1 min intervals for 3 min.

Total protein level

Total protein was assayed by the method of Bradford (1976) [29], where Coomassie Brilliant Blue dye reacted with Bradford reagent and gave a blue complex at λ_{max} 595 nm.

Histopathological analysis

Liver slices were fixed in 10% paraformaldehyde and embedded in paraffin wax blocks. Sections of 5 μm thick were stained with hematoxylin & eosin (H&E) and Masson's trichrome, then examined under light microscope for determination of pathological changes [30].

Statistical analysis

All data were expressed as mean \pm S.D. of eight rats in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program.

Results

Phytochemical study

Phytochemical investigation of the plant aerial parts resulted in the isolation of one flavanone and eight flavones. Their spectral data were illustrated as the following:

Compound (1): $\text{C}_{15}\text{H}_{12}\text{O}_4$; 24 mg; R_f : 0.83 (S_1), 0.73 (S_2); white needles; 197-198°C; UV spectral data: λ_{max} , nm (MeOH): 232 sh, 290, 338 sh; (+NaOMe): 247, 328; (+ AlCl_3): 312, 377; (+ AlCl_3/HCl): 310, 377; (+NaOAc): 232, 332; (+NaOAc/ H_3BO_3): 236, 293, 336 sh; MS: m/z (%): 256 (M^+ , 41.8), 257 [($M+H$) $^+$, 7.8], 255 [($M-H$) $^+$, 44.3], 238 [($M-H_2O$) $^+$, 6.5], 179 [($M-C_6H_5$) $^+$, 55.7], 153 [A_1^+H , 15.6], 152 [A_1^+ , 53.9], 124 [$A_1^+ - CO$, 58.2], 104 [B_1^+ , 34.8], 77 [$C_6H_5^+$, 81.6], 51 [$C_6H_5^+ - C_2H_2$, 100]; $^1\text{H NMR}$ (DMSO- d_6 , 300 MHz): δ ppm 12.12 (s, OH-5), 7.38-7.50 (5H, m, H-2'/3'/4'/5'/6'), 5.92 (1H, d, J=2 Hz, H-8), 5.90 (1H, d, J=2 Hz, H-6), 5.57 (d, J=12.6 Hz, H-2), 3.24 (1H, dd, J=12.6, 16.8 Hz, H-3), 2.78 (1H, d, J=17.1 Hz, H-3); $^{13}\text{C NMR}$ (DMSO- d_6 , 75 MHz): δ ppm 195.8 (C-4), 166.7 (C-7), 163.4 (C-5), 162.6 (C-9), 138.6 (C-1'), 128.5 (C-3'/4'/5'), 126.5 (C-2'/6'), 101.7 (C-10), 95.9 (C-6), 95.0 (C-8), 78.3 (C-2), 42.1 (C-3) (Fig.1).

Compound (2): $\text{C}_{18}\text{H}_{16}\text{O}_7$; 32 mg; R_f : 0.72 (S_1), 0.76 (S_2); yellow ppt, 161-162°C; UV spectral data: λ_{max} , nm (MeOH): 273, 343; (+NaOMe): 278, 297, 371; (+ AlCl_3): 281, 305 sh, 360, 409; (+ AlCl_3/HCl): 283, 305 sh, 354, 409 sh; (+NaOAc): 273, 299 sh, 367; (+NaOAc/ H_3BO_3): 273, 343; MS m/z (%): 344 [M^+ , 100], 345 [($M+H$) $^+$, 35.6], 330 [$M^+ - \text{CH}_3/H$, 19.4], 329 [$M^+ - \text{CH}_3$, 58.3], 301 [$M^+ - \text{CO}/\text{CH}_3$, 48.2], 283 [301- H_2O , 25.0], 258 [301 - CO/CH_3 , 11.0], 135 [B_2^+ , 18.5], 132 [B_1^+ , 6.4]; $^1\text{H NMR}$ (DMSO- d_6 , 300 MHz): δ ppm 12.70 (1H, s, OH-5), 7.93 (2H, d, J=8.7 Hz, H-2'/6'), 7.03 (2H, d, J=8.7 Hz, H-3'/5'), 6.49 (1H, s, H-8), 3.81 (3H, s, OCH_3 -6), 3.76 (3H, s, OCH_3 -3), 3.72 (3H, s, OCH_3 -4'); $^{13}\text{C NMR}$ (DMSO- d_6 , 75 MHz): δ ppm 178.1 (C-4), 161.2 (C-4'), 157.2 (C-7), 155.0 (C-2), 152.3 (C-5), 151.5 (C-9), 137.5 (C-3), 131.1 (C-6), 129.7 (C-2'/6'), 122.2 (C-1'), 114.0 (C-3'/5'), 104.6 (C-10), 93.9 (C-8), 59.8 (OCH_3 -6), 59.6 (OCH_3 -3), 55.3 (OCH_3 -4') (Fig.1).

Compound (3): $\text{C}_{16}\text{H}_{11}\text{O}_6$; 13 mg; R_f : 0.60 (S_4), 0.16 (S_5); m.p. 237-242°C; yellow needle crystals; UV spectral data: λ_{max} , nm (MeOH): 266, 297 sh, 348; (+NaOMe): 274, 324 sh, 398; (+ AlCl_3): 274, 305 sh, 350 sh, 399; (+ AlCl_3/HCl): 274, 305sh, 347 sh, 399. (+ NaOAc): 274, 309 sh, 386; (+ NaOAc/ H_3BO_3): 266, 303sh, 350; MS: m/z (%): 300 (M^+ , 100), 299 ($M^+ - H$, 89), 282 ($M^+ - \text{H}_2\text{O}$, 37), 257 ($M^+ - \text{CH}_3\text{CO}$, 73); $^1\text{H NMR}$ (DMSO- d_6 , 300 MHz): δ ppm 9.2 (2H, d, J=9 Hz, H-2'/6'), 6.93 (2H, d, J=9 Hz, H-3'/5'), 6.37 (1H, d, J=2.1 Hz, H-8), 6.14 (1H, d, J=2.1 Hz, H-6), 3.77 (3H, s, OCH_3 -3).

¹³C NMR (DMSO-d₆, 75 MHz): δ ppm 177.8 (C-4), 164.0 (C-7), 161.1 (C-9), 160.0 (C-4'), 156.3 (C-5), 155.5 (C-2), 137.5 (C-3), 129.9 (C-2'/6'), 120.5 (C-1'), 115.5 (C-3'/5'), 104.1 (C-10), 98.5 (C-6), 93.6 (C-8), 59.6 (OCH₃-3) (Fig.1).

Compound (4): C₁₆H₁₁O₆; 12 mg; R_f: 0.68 (S₁), 0.51(S₂); yellow powder, 224-225°C; UV spectral data: λ_{max}, nm (MeOH): 270, 363; (+NaOMe): 278, 399; (+AlCl₃): 274, 305 sh, 348, 424; (+AlCl₃/HCl): 274, 305 sh, 348, 424; (+NaOAc): 276, 364; (+NaOAc/H₃BO₃): 276, 364; ¹H NMR (DMSO-d₆, 300 MHz): δ ppm 12.45 (1H, s, OH-5), 8.03 (2H, d, J=8.7 Hz, H-2'/6'), 6.92 (2H, d, J=8.7 Hz, H-3'/5'), 6.44 (1H, d, J=2.4 Hz, H-8), 6.19 (1H, d, J=2.1 Hz, H-6), 3.46 (3H, s, OCH₃-7). ¹³C NMR (DMSO-d₆, 75 MHz): δ ppm 176.0 (C-4), 164.0 (C-7), 160.8 (C-5), 159.3 (C-4'), 156.4 (C-9), 147.2 (C-2), 135.8 (C-3), 129.7 (C-2'/6'), 121.8 (C-1'), 115.6 (C-3'/5'), 106.0 (C-10), 98.4 (C-6), 93.7 (C-8), 57.0 (OCH₃-7) (Fig.1).

Compound (5): C₁₆H₁₂O₇; 19 mg, R_f: 0.52 (S₁), 0.36 (S₂); yellow powder, 314-415°C; UV spectral data: λ_{max}, nm (MeOH): 254, 300 sh, 374; (+NaOMe): 274, 321, 412; (+AlCl₃): 265, 305 sh, 353, 428; (+AlCl₃/HCl): 265, 305 sh, 353, 428; (+NaOAc): 257 sh, 274, 331, 388; (+ NaOAc/H₃BO₃): 254, 274 sh, 374; MS: m/z (%): 316 (M⁺, 100), 301 (M⁺- CH₃, 9.9), 288 (M⁺-CO, 4.9), 287 (M⁺-CHO, 10.2), 273 (C₁₄H₉O₆⁺, 7.2), 245 (C₁₃H₉O₅⁺, 10.1), 153 [(A₁+H)⁺, 6.2], 151 (B₂⁺, 5.9); ¹H NMR (DMSO-d₆, 300 MHz): δ ppm 12.44 (1H, s, OH-5), 7.77 (1H, dd, J=1.2, 8.7 Hz, H-6'), 7.69 (1H, d, J=1.2 Hz, H-2'), 6.93 (1H, d, J=8.7 Hz, H-5'), 6.46 (1H, s, H-8), 6.18 (1H, s, H-6), 3.92 (3H, s, OCH₃-3'); ¹³C NMR (DMSO-d₆, 75 MHz): δ ppm 176.3 (C-4), 164.4 (C-7), 161.2 (C-5), 156.8 (C-9), 149.4 (C-3'), 147.1 (C-4'), 147.1 (C-2), 136.1 (C-3), 122.6 (C-1'), 122.2 (C-6'), 116.0 (C-5'), 112.6 (C-2'), 103.5 (C-10), 98.6 (C-6), 93.4 (C-8), 55.7 (OCH₃-3') (Fig.1).

Compound (6): C₁₅H₁₀O₇; 24 mg, R_f: 0.61 (S₄), 0.25 (S₅); yellow powder; m.p.: 316-318°C; UV spectral data: λ_{max}, nm (MeOH): 258, 300 sh, 372; (+NaOMe): 274, 325, 410; (+AlCl₃): 272, 330 sh, 449; (+AlCl₃/HCl): 265, 305 sh, 357, 430; (+NaOAc): 257, 378; (+ NaOAc/H₃BO₃): 363, 388; MS: m/z (%): 302 [M⁺, 100], 301 [(M-H)⁺, 24.0], 273 [M⁺-CHO, 4.0], 245 [M⁺-CHO/CO, 8.0], 153 [(A₁+H)⁺, 4.0], 137 [B₂⁺, 9.4]; ¹H NMR (DMSO-d₆, 300 MHz): δ ppm 12.47 (1H, s, OH-5), 7.67 (1H, d, J=2.1 Hz, H-2'), 7.53 (1H, dd, J=2.1, 8.7 Hz, H-6'), 6.88 (1H, d, J=8.7 Hz, H-5'), 6.41 (1H, d, J=1.5 Hz, H-8), 6.18 (1H, d, J=1.5 Hz, H-6); ¹³C NMR (DMSO-d₆, 75 MHz): δ ppm 176.3 (C-4), 164.3 (C-7), 161.1 (C-5), 156.7 (C-9), 148.1 (C-2), 147.4 (C-4'), 145.5 (C-3'), 136.1 (C-3), 122.5 (C-1'), 120.7 (C-6'), 116.1 (C-5'), 115.5 (C-2'), 103.5 (C-10), 98.8 (C-6), 94.0 (C-8) (Fig.1).

Compound (7): C₁₇H₁₄O₇; 34 mg, R_f: 0.47 (S₁), 0.55 (S₂); yellow crystals; m.p. 210-212°C; UV spectral data: λ_{max}, nm (MeOH): 272, 342; (+NaOMe): 277, 329, 400; (+AlCl₃): 278, 307 sh, 360, 406 sh; (+AlCl₃/HCl): 283, 307 sh, 360, 406 sh; (+NaOAc): 273, 369; (+NaOAc/H₃BO₃): 273, 301 sh, 342; MS: m/z (%): 330 [M⁺,

100], 329 [(M-H)⁺, 42.7], 316 [M⁺-CH₃/+H, 12.2], 315 [M⁺-CH₃, 47.9], 312 [(M-H₂O)⁺, 42.0], 287 [M⁺-CH₃/CO, 47.3], 269 [287-H₂O, 32.9], 167 [A₁⁺, 8], 121 [B₂⁺, 36.2]; ¹H NMR (DMSO-d₆, 300 MHz): δ ppm 12.75 (1H, s, OH-5), 7.92 (2H, d, J=8.7 Hz, H-2'/6'), 6.93 (2H, d, J=8.7 Hz, H-3'/5'), 6.53 (1H, s, H-8), 3.77 (s, 3H, OCH₃-6), 3.75 (3H, s, OCH₃-3); ¹³C NMR (DMSO-d₆, 75 MHz): δ ppm 178.1 (C-4), 160.1 (C-4'), 157.2 (C-7), 155.6 (C-2), 152.3 (C-5), 151.5 (C-9), 137.2 (C-3), 131.2 (C-6), 130.0 (C-2'/6'), 120.6 (C-1'), 115.6 (C-3'/5'), 104.5 (C-10), 93.9 (C-8), 59.9 (OCH₃-6), 59.6 (OCH₃-3) (Fig.1).

Compound (8): C₁₇H₁₄O₇; 19 mg; R_f: 0.92 (S₄), 0.13 (S₅); yellowish white amorphous; UV spectral data: λ_{max}, nm (MeOH): 257, 270 sh, 346; (+NaOMe): 269, 300 sh, 408; (+AlCl₃): 270, 280 sh, 300 sh, 352, 400 sh; (+AlCl₃/HCl): 263, 270 sh, 356, 390 sh; ¹H NMR (CDCl₃, 300 MHz): δ ppm 7.64 (1H, d, J=1.9 Hz, H-2'), 7.60 (1H, dd, J=8.7, 1.9 Hz, H-6'), 6.99 (1H, d, J=8.7 Hz, H-5'), 6.44 (1H, s, H-8), 3.92, 3.89, 3.85, 3.79 (3H, s each, 4x OCH₃); ¹³C NMR (CDCl₃, 75 MHz): δ ppm 178.7 (C-4), 158.6 (C-7), 155.8 (C-2), 152.6 (C-9), 152.1 (C-5), 148.2 (C-4'), 146.2 (C-3'), 138.5 (C-6), 132.1 (C-3), 122.5 (C-6'), 122.3 (C-1'), 114.5 (C-5'), 110.8 (C-2'), 106.5 (C-10), 90.3 (C-8), 60.9 (OCH₃-6), 60.2 (OCH₃-3), 56.3 (OCH₃-3'), 56.1 (OCH₃-4') (Fig.1).

Compound (9): C₂₂H₃₂O₁₆; R_f: 0.12 (S₁), 0.15 (S₂); 20 mg, yellow granules, m.p. 190-191°C; HRMSI MS m/z: 625 [M+H]⁺, 479 [M⁺-Rha], 317 [aglycone+H]⁺, -ve MS: 623 [M-H]⁺; ¹H NMR (DMSO-d₆, 300 MHz): δ ppm 8.01 (1H, d, J=2.1 Hz, H-2'), 7.59 (1H, dd, J=2, 8.7 Hz, H-6'), 6.90 (1H, d, J=8.4 Hz, H-5'), 6.37 (1H, d, J=2.1 Hz, H-8), 6.18 (1H, d, J=2.1 Hz, H-6), 5.19 (1H, d, J=7.8 Hz, H-1''), 4.95 (1H, br s, H-1''), 3.96 (3H, s, OCH₃-3'), 3.8-3.3 (m, remaining sugar moiety), 1.17 (3H, d, J=6.3 Hz, CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ ppm 177.4 (C-4), 164.5 (C-7), 161.2 (C-5), 156.5 (C-9), 156.4 (C-2), 147.0 (C-4'), 149.5 (C-3'), 132.9 (C-3), 122.0 (C-6'), 121.2 (C-1'), 115.2 (C-5'), 113.6 (C-2'), 104.1 (C-10), 101.8 (C-1'), 100.1 (C-1'''), 98.8 (C-6), 93.8 (C-8), 73.6 (C-5''), 73.0 (C-3''), 71.9 (C-4''), 71.1 (C-2''), 70.6 (C-3''), 70.4 (C-2''), 68.3 (C-5''), 68.0 (C-4''), 65.2 (C-6''), 55.9 (OCH₃-3'), 18.0 (C-6''') (Fig.1).

Bioactivity study

In vitro antioxidant activity

In vitro antioxidants screening of *D. viscosa* ethanol extract, its toluene and ethyl acetate fractions as well as the flavanone (1) and major flavones (2, 5, and 7-9) was carried out. All tested samples scavenge DPPH free radicals by variable degrees ranging from 12.50% to 80.00% (Table 1). The ethanol extract recorded the highest inhibition level of DPPH free radicals (80.00%) than the fractions of toluene (68.75%) and ethyl acetate (46.00%) (Table 1). Compound 5 showed remarkable antioxidant activity compared to the other tested flavones.



Table 1 Inhibition percentage of DPPH radicals after treatment with *D. viscosa* samples.

Sample	DPPH inhibition(%)
Ethanol extract	80.00
Toluene extract	68.75
Ethyl acetate extract	79.00
Compound (1)	75.00
Compound (2)	46.00
Compound (5)	80.00
Compound (6)	96.40
Compound (7)	61.14
Compound (8)	48.07
Compound (9)	12.50

Percentage inhibition (%) = $\frac{A \text{ control} - A \text{ sample}}{A \text{ sample}} \times 100$

Oxidative stress markers

Malondialdehyde (MDA) showed significant increase in CCl₄ group by 40% (Table 2). Treated group with ethyl acetate extract still recorded significant increase (22.66%) as compared with normal control group, while treatment with ethanol, toluene and silymarin showed insignificant changes. Treatment with ethanol extract showed the most improvement in MDA level (36.00%) comparing with the reference drug silymarin (38.67%) (Table 2). CCl₄ group showed significant decrease in glutathione (62.94%) and superoxide dismutase (49.33%). Treatment with different extracts and silymarin recorded significant decrease in GSH and SOD. Glutathione showed significant decrease by 16.85, 27.21, 39.33 and 21.89% after treatment with ethanol, toluene, ethyl acetate and silymarin, respectively, while SOD decreased by 28.00, 38.66, 40.80 and 28.00%, respectively (Table 2). Treatment with ethanol extract showed the most potent improvement in GSH (46.09%) and SOD (21.33%) levels (Table 3).

Table 2 Effect of different extracts on hepatic oxidative stress markers in CCl₄ treated rats.

Parameters	Control	CCl ₄	CCl ₄ treated with ethanol extract	CCl ₄ treated with toluene extract	CCl ₄ treated with ethyl acetate extract	CCl ₄ treated with silymarin
Malondialdehyde (MDA)	0.75±0.03 ^c	1.06±0.12 ^a	0.78±0.05 ^c (36.00)	0.88±0.02 ^{bc} (17.34)	0.92±0.12 ^b (22.67)	0.76±0.02 ^c (38.67)
Glutathione (GSH)	769.75±15.10 ^a	285.25±14.78 ^f	640.00±30.14 ^b (46.09)	560.25±19.68 ^d (35.73)	467.00±12.88 ^e (23.61)	601.25±41.69 ^c (41.05)
Super oxide dismutase (SOD)	18.75±2.50 ^a	9.50±1.29 ^c	13.50±1.22 ^b (21.33)	11.50±1.29 ^{bc} (10.67)	11.10±0.81 ^{bc} (8.53)	13.50±1.29 ^b (21.33)

Data are means ± SD of eight rats in each group.

Data are expressed as µg/mg protein for GSH and µmol/mg protein for MDA and SOD.

Superscript unshared letters between groups are the significance values at p < 0.0001.

Statistical analysis is carried out using one way analysis of variance (ANOVA), CoStat Computer Program.

Values between brackets are improvement percentage (%) = $\frac{\text{Mean of treated group} - \text{Mean of CCl}_4 \text{ group}}{\text{Mean of control group}} \times 100$

Table 3 Effect of different extracts on liver function enzymes and serum protein level in CCl₄ treated rats.

Parameters	Control	CCl ₄	CCl ₄ treated with ethanol extract	CCl ₄ treated with toluene extract	CCl ₄ treated with ethyl acetate extract	CCl ₄ treated with silymarin
AST	17.75±1.70 ^c	27.00±2.58 ^a	19.75±1.71 ^{bc} (40.84)	21.80±2.16 ^{bc} (29.29)	25.50±2.08 ^a (8.45)	21.25±2.1 ^{bc} (32.40)
ALT	34.58±2.67 ^c	51.25±2.98 ^a	35.50±3.41 ^c (45.54)	38.00±4.32 ^{bc} (38.31)	43.25±3.89 ^b (23.13)	40.50±2.08 ^{bc} (31.09)
ALP	12.25±1.70 ^c	23.00±2.58 ^a	14.75±1.25 ^{bc} (67.35)	15.00±0.81 ^{bc} (65.31)	17.25±1.25 ^b (46.94)	15.50±1.63 ^{bc} (61.22)
GGT	15.75±1.73 ^b	26.00±1.63 ^a	14.75±1.70 ^b (65.07)	18.50±1.29 ^b (58.73)	18.75±0.95 ^b (47.61)	17.80±1.29 ^b (46.03)
Serum protein	14.00±1.41 ^b	21.25±2.50 ^a	15.75±1.70 ^b (39.28)	16.50±1.29 ^b (33.93)	16.72±1.29 ^b (32.36)	14.75±1.25 ^b (46.43)

• Data are means ± SD of eight rats in each group.

• Liver functions are expressed as Unit/L. Total protein is expressed as mg P/ ml.

• Superscript unshared letters between groups are the significance values at p < 0.0001.



- Statistical analysis is carried out using one way analysis of variance (ANOVA), CoStat Computer Program.
- Values between brackets are improvement percentages (%) = $\frac{\text{Mean of treated group} - \text{Mean of CCl}_4 \text{ group}}{\text{Mean of control group}} \times 100$

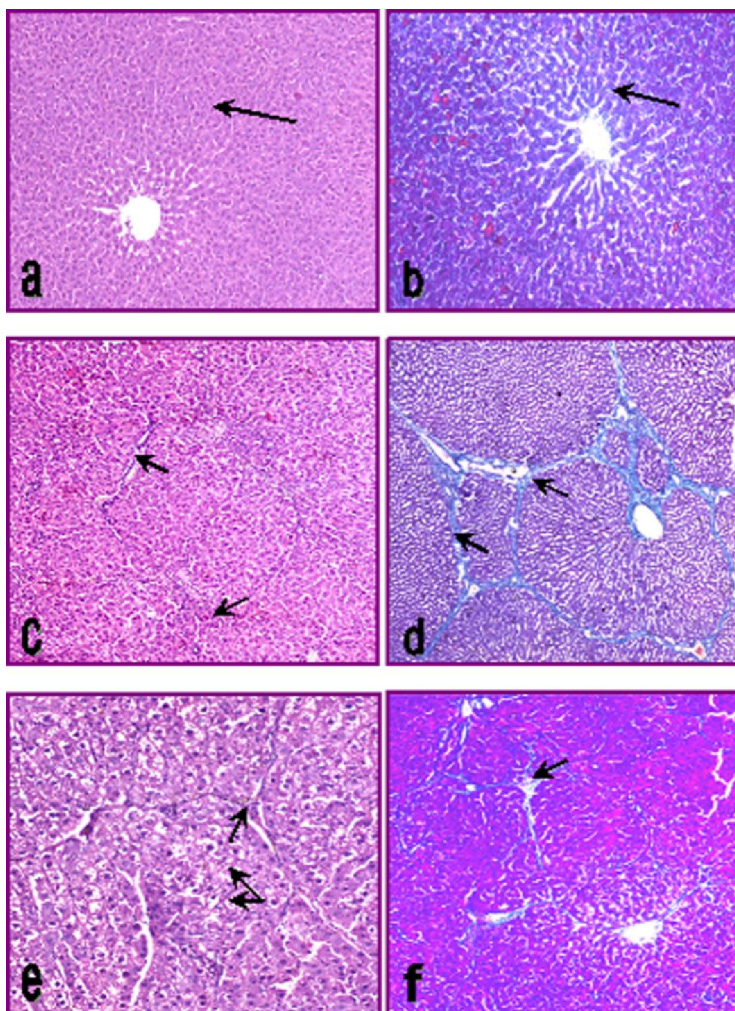


Fig.2: Photomicrograph of hematoxylin and eosin (H & E) and Masson's trichrome stained liver section (100x) of control (a,b), CCl₄ (c,d) and silymarin (e,f) treated groups. Arrows indicate well arranged hepatocytes. Small arrows indicate collagen deposition and swollen hepatocytes.

Liver function enzymes

In case of liver function enzymes, CCl₄ group recorded significant increase in AST, ALT, ALP and GGT by 52.11, 48.20, 87.75 and 65.07%, respectively. Treatment with ethanol and toluene extracts showed insignificant increase in liver function enzymes as compared with the normal control group. Treatment with ethyl acetate extract still recorded significant increase in AST, ALT and ALP by 43.66, 25.07 and 40.81%, respectively. Treatment with silymarin showed insignificant increase in liver function parameters (Table 3). Highly improvement levels in liver functions were

recorded after treatment with ethanol extract. It reached to 40.84, 45.54, 67.35 and 47.61% for AST, ALT, ALP and GGT, respectively (Table 3).

Serum total protein level

CCl₄ group recorded significant increase by 51.78%. Insignificant changes were observed after treatment with plant extracts and silymarin as compared to normal control group (Table 3). Ethanol

extract showed the highest amelioration in protein level (39.28%) than the other extracts, while silymarin treatment recorded improvement by 46.43% (Table 3).

Liver histopathological analysis

Liver section of control healthy rats showed normal hepatic lobular architecture (Figure 2 a,b). The hepatocytes were within normal limits and arranged in thin plates. No fibrous tissue or lymphocytes

deposition were observed. Liver injured with CCl_4 revealed portal loss of hepatic lobular architecture. Ballooning of hepatocytes, deformed cord arrangement and disturbed sinusoids were seen. Portal tracts were extended with marked number of chronic inflammatory cells and fibrous tissue (Fig. 2 b,c). CCl_4 group treated with silymarin showed swelling and foamy appearance of hepatocytes. Hydropic changes were also seen. Mild fibrotic tissue was still present (Figure 2e,f).

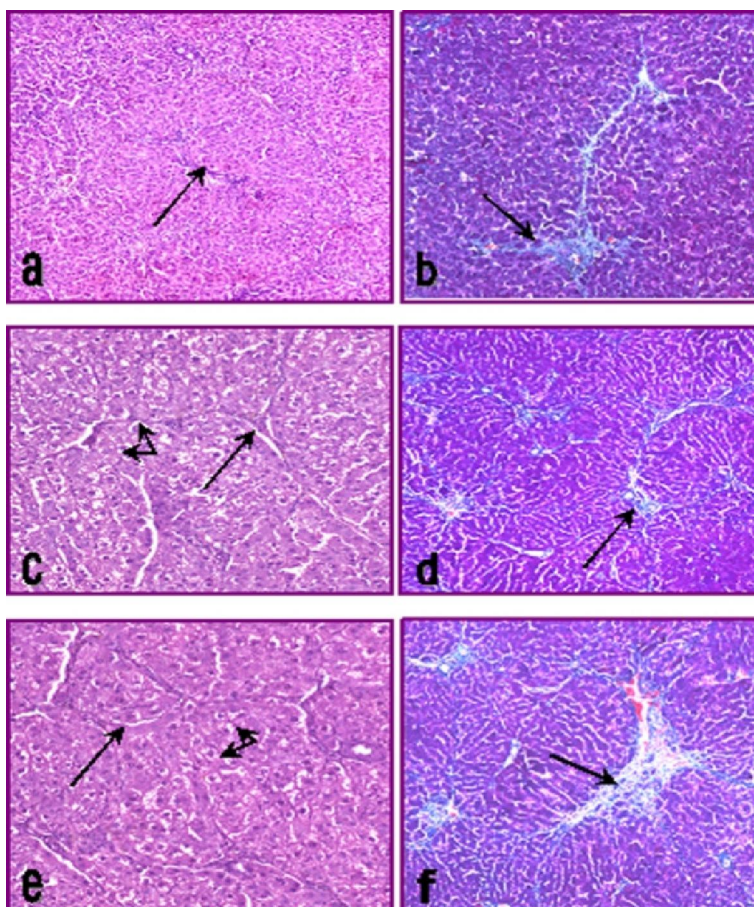


Fig.3: Photomicrograph of hematoxylin and eosin (H & E) and Masson's trichrome stained liver section (100x) of CCl_4 group treated with ethanol (a,b), toluene (c,d) and ethyl acetate (e,f) extracts. Arrows indicate collagen deposition. Small arrows indicate swollen hepatocytes.

Treatments of injured liver with the ethanol extract showed well formed nucleated hepatocytes. Mild inflammatory lymphocyte infiltration and mild fibrosis were seen (Figure 3 a,b). Toluene extract partly preserved the hepatic architecture with mild degree of hydropic changes. The hepatocytes were still swollen with narrow sinusoids. Portal tracts were extended with mild fibrous tissue (Figure 3 c,d). Ethyl acetate extract showed ballooning of hepatocytes that lost its arrangement with narrow sinusoides due to hepatocytes enlargement (Figure 3 e). Moderate fibrous tissue was recorded (Figure 3 f).

Discussion

In the present study, the known flavones 2-9 as well as flavanone 1 were isolated. The identification of the isolated flavones was confirmed by MS, UV and NMR spectra as well as co-chromatography with available authentic samples.

Compound 1 showed λ_{max} 232, 290, and 328 nm in methanol characteristic for flavanone structure. The $^1\text{H-NMR}$ spectrum showed a two proton aromatic signals doublets at δ 5.92 and 5.90 ppm (J - 2 Hz) for H-8 and H-6, respectively. A

monosubstituted aromatic ring and a CH-CH₂- system [5.57 ppm (d, J=12.6 Hz, H-2), 3.24 ppm (dd, J=12.6, 16.8 Hz, H-3) and 2.78 ppm (d, J=17.1 Hz, H-3)], thus suggesting a flavanone skeleton. ¹³C NMR showed resonance of 15 carbons in which C-3 appeared at δ 42.1 ppm and C-2 at δ 78.3 ppm. C-7 and C-5 appeared at downfield of δ 166.7 and 163.4 ppm, respectively so they occupied by free OH. The spectral data of ¹H-¹H COSY, HMQC and HMBC supported the structure of 1 as 5,7-dihydroxy flavanone (pinocembrin) [16, 31].

UV spectral data of compounds 2 - 9 showed two absorptions at λ_{max} the range of 250-270 nm of band II and another at the range of 330-350 nm of band I indicated that they may be 3-substituted flavonol or flavone [18].

The UV data were in agreement with the presence of a free 4'-hydroxyl group in compounds 3-7 and 9 as shown by the strong increase in band I with NaOMe, while 2 and 8 showed a diminished peak confirming a substituted 4'-OH.

Absence of bathochromic shift in band II after addition NaOAc reagent indicated a substituted 7-OH group in the structures of all compounds, except compound 7.

The lack of bathochromic shift in NaOAc/H₃BO₃ spectrum indicates the absence of orthodihydroxy group in ring B in all compounds, except compound 6. The presence of orthodihydroxy group in compound 6 was proved also by the observed hypsochromic shift in band I with AlCl₃/HCl as a shift reagent.

The AlCl₃ spectrum showed a relatively large bathochromic shift in all compounds indicating the existence of OH-5 and/or OH-3.

¹H NMR spectra of compounds 2-9 showed correlation to each other of flavones structure. The most downfield shifted peak was at δ 175.8-179.3 ppm, which was assigned to ketone group C-4.

Two ¹³C NMR at δ 129.7-130.0 ppm and δ 114.0-115.6 ppm, which were attached directly to the ¹H peaks at δ 7.92-8.03 ppm and δ 6.92-7.03 ppm, respectively, corresponding to C-2'/6' and C-3'/5' for compounds 2-4 and 7 [1, 19, 32, 33].

The single proton singlet at δ 6.37-6.53 ppm was assigned to H-8 in all the isolated compounds.

The presence of a methoxyl group at C-6 could be deduced from the corresponding signals at δ 131.1, 132.1 and 132.1 ppm of compounds 2, 7 and 8 respectively.

A low field of C-5 and C-7 at δ 152.1 -152.3 ppm and δ 157.2-158.7 ppm in the spectrum of compounds 2, 7 and 8, respectively, again indicated the presence of a methoxyl group. Furthermore, the downfield shifts of C-3 in compounds 2, 3, 7 and 8 at δ 137.5, 137.5, 137.2 and 138.5 ppm, respectively.

Galactose and rhamnose signals were observed in the ¹³C NMR spectrum of 9. Two anomeric proton signals at 5.19 (d, J = 7.8 Hz, H-1") and 4.95 (brs, H-1") observed in the ¹H NMR spectrum.

The glycosylation site at C-3 hydroxyl of compound 9 was confirmed through the downfield resonance of C-2 at δ 156.4 ppm and the upfield signal of C-3 at δ 132.9 ppm.

The downfield shift of about 4.9 ppm for C-6" and an upfield shift of about 2.0 ppm for C-5" of galactose further was indicated the interglycosidic linkage at C-6" of galactose. These results revealed the internal sugar was galactose and external sugar was rhamnose. On the basis of these data and comparison with the reported NMR data [34, 35] the structure of the compound 9 was identified as isorhamnetin 3-O- -D-(6-O- -L-rhamnosyl) galactoside (isorhamnetin 3-robinobioside).

The known compounds were to be 5,7-dihydroxy flavanone (pinocembrin) (1) [1, 31, 36] and eight flavones were isolated and elucidated as following; 5,7-dihydroxy-3,6,4'-trimethoxyflavone (santini) (2) [19, 32], 5,7,4'-trihydroxy-3-methoxy flavone (kaempferol 3-O-methyl ether) (3), 3,4',5-trihydroxy-7-methoxy flavone (kaempferol 7-O-methyl ether) (4), 3',4',5,7-tetrahydroxy-3-methoxy-flavone (quercetin 3'-O-methyl ether) (5) [16], 3,3',4',5,7-pentahydroxyflavone (quercetin) (6) [16], 5,7,4'-trihydroxy-3,6-dimethoxy flavone (7) [1, 32, 33], 5,7-dihydroxy 3,6,3',4'-tetramethoxy flavone (8) and isorhamnetin-3-O-robinobioside (9) [34, 35].

The previously isolated compounds from *D. viscosa* were; compound of 1 and 9 [36], 2 [33], 5 and 6 [37], and 7 [33, 38]. While in the present study; compound of 3, 4, and 8 were isolated for the first time from *Dodonaea* species. Compounds 3 - 5 are methyl ethers known as a group of rare naturally occurring compounds.

The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Compound 6 (3,5,7,3',4'-pentahydroxyflavone) showed the highest antioxidant activity. Antioxidant activity usually increased with an increase in the number of hydroxyl groups as previously reported [39]. Also, compound 5 (3'-methoxy-3',4',5,7-tetrahydroxyflavone) showed higher antioxidant activity than compound 7 (5,7,4'-trihydroxy-3,6-dimethoxy flavone). The last compound showed higher antioxidant activity compared with compound 8 (5,7-dihydroxy 3,6,3',4'-tetramethoxy flavone). The results are in agreement with the study of Fukumoto and Mazz (2000) [39]. They reported that compounds with three hydroxyl groups on the B ring of flavonoids had high antioxidant activity. The loss of one hydroxyl group decreased activity slightly, whereas the loss of two hydroxyl groups significantly decreased activity [39]. Antioxidant activity usually increased with a decrease in glycosylation. The current study showed higher activity of compound 5 compared to its glycoside derivative of compound 9. The hydroxyl group is present in compound 5 while it was substituted in compounds of 7-9. Flavonoids with 3-OH group play a positive role in antioxidant activities [40]. It is believed that these structure activity relationship results can be taken into account for the development of flavonoids with high therapeutic index. It appears that methylation of 3-OH in compound 5 reduced activity when compared with compound 2 with free 3-OH. This observation is in agreement with the earlier structural activity study [41, 42].

Due to extensive methoxylation (3-OMe, 4'-OMe and 6-OMe) of compound 2 and 8, they may demonstrate less activity than the

other compounds. This observation suggests that 5-OH does not play a strong role in antioxidant activity of the investigated compounds. This is also in line with the earlier report on flavonoids [43]. It was also observed the slight higher activity of compound **2** than **8** may related to the excess 3'-OMe of the last compound.

Carbon tetrachloride is a toxic compound used extensively for enhancement of oxidative stress [10]. Many studies have established the fact that CCl₄ is metabolized in the liver into a highly reactive trichloromethyl, which initiate free radicals that mediate lipid peroxidation [11] and generate ROS_s [12]. However, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient. Therefore, ROS affects the antioxidant defense mechanisms, reduces the intracellular concentration of glutathione (GSH), decreases the activity of superoxide dismutase (SOD) and enhances lipid peroxidation (LPO) [44]. This was confirmed by the observed reduction in GSH and the increase in malondialdehyde after CCl₄ intoxication. The decline in the activity of free radical scavenging enzyme SOD may be due to its inactivation caused by excess reactive oxygen species production. SOD neutralizes superoxide; that cannot cross lipid membrane, then produce hydrogen peroxide and cross biological membranes. So, reduction in SOD may damage the first line of enzymatic defense against superoxide anion and hydrogen peroxide [45]. The significant depletion of GSH indicates damage to the second line of antioxidant defense. Gharib et al. (1999) [46] attributed the decrease in glutathione level to increased cytotoxicity by H₂O₂ which is produced as a result of inhibition of glutathione reductase that keep glutathione in its reduced form.

Treatment with *D. viscosa* extracts normalized the antioxidant levels through their rich of compounds of kaempferol and its methyl ether derivatives using DPPH free radicals technique [43]. Silymarin as an antioxidant flavonoid complex was reported to have the ability to attenuate free radicals elevation, chelates metal ions, inhibits lipid peroxidation and prevents liver glutathione depletion [47].

ALT and AST had been reported to be sensitive indicators of liver injury [48]. Significant elevation of ALT and AST after CCl₄ intoxication was in agreement with the reported studies [12, 49]. In addition, Romero et al. (1998) [50] showed that CCl₄ intoxication induced changes in the process of protein synthesis. Hence, increase in total protein content can be deemed as a useful index of the severity of cellular dysfunction in liver diseases as clearly shown in our studies. Stimulation of protein synthesis has been advanced as a contributory self healing mechanism, which accelerates liver regeneration process [51]. Increment of ALP and GGT were also observed in the present study. This was in agreement with Reyes-Gordillo et al. (2007) [52] who recorded significant increase in ALP and GGT biomarker after intoxication of rats with CCl₄. Hamed (2011) [53] added that GGT alone is a poor indicator of cytotoxicity and suggested the combination of other markers like AST, ALT and LDH for accurate detection and early diagnosis. Serum enzymes

elevation can be explained to the increase in hepatic cell membrane fluidity that led to enzyme release into circulation [12, 49]. Treatment with *D. viscosa* extracts attenuated the increased level of serum enzymes and caused a subsequent recovery towards normalization. This give an additional support that *D. viscosa* extracts are able to condition the hepatocytes, accelerate regeneration of parenchyma cells, protect against membrane fragility and decrease leakage of the enzymes into circulation. Therefore, plant extracts acted by the same mode of action of silymarin [12]

The in vitro screening of the plant extracts confirmed the in vivo study, where ethanol extract recorded the highest percentage in scavenging DPPH free radicals revealing its high antioxidant effect followed by toluene extract.

The most remarkable histopathological characteristics of CCl₄-induced hepatotoxicity are massive centrilobular necrosis, ballooning degeneration, cellular infiltration and steatosis [12]. This was in accordance with the present finding of massive deformation of hepatic cells architecture. In *D. viscosa* and silymarin treated groups, hepatocyte degeneration, necrosis and infiltration of inflammatory cells were all apparently ameliorated. We compare the anti-fibrogenic effects of silymarin with extracts and the results exhibited that ethanol extract had higher potency in inhibiting collagen deposition and fibrosis severity.

Any curative abilities of *D. viscosa* is most likely associated with the pharmacological effects brought by the synergistic combination of several compounds, namely, saponins, flavonoids, di- and triterpenes, and a complex mixture of phenolic compounds present in the plant [54]. Previous phytochemical investigations of *Dodonaea viscosa* have resulted in the isolation of flavonoids [1-4], saponins, and diterpenes [2]. Treatment with the flavonoidal drug (*D. viscosa*) against lead-poisoned rats exhibited a significant decrease in the levels of glycoproteins and sialic acid contents in the liver [55].

Conclusions

Dodonaea viscosa has the ability to down regulate free radicals elevation, ameliorate hepatic function enzymes, reduce fibrosis severity and normalize the hepatic cells architecture. *Dodonaea viscosa* ethanolic extract recorded the most potent effect in improving the selected parameters. Further in vivo studies are required in order to identify the molecules responsible of the pharmacological activity in the hope to can use this species (crude extract, fractions, sub fractions or pure compounds) in the therapy of liver fibrosis and in a clinical trial for its use as a complementary drug capable to attenuate liver fibrotic mechanism or reduce disease complication.

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References

- [1]. van-Heerden FR, Viljoen AM, van-Wyk BE. The major flavonoid of *Dodonaea angustifolia*. *Fitoterapia* 2000;71:602–604.
- [2]. Niu HM, Zeng DQ, Long CL, Peng YH, Wang YH, Luo JF, Wang HS, Shi YN, Tang GH, Zhao FW. Clerodane diterpenoids and prenylated flavonoids from *Dodonaea viscosa*. *J Asian Nat Prod Res* 2010;12:7–14.
- [3]. Shanmugavasana A, Ramachandran T. Investigation of the extraction process and phytochemical composition of preparations of *Dodonaea viscosa* (L.) Jacq. *J Ethnopharmacol* 2011;137:1172-1176.
- [4]. Pengelly A. Medicinal activity of *Dodonaea viscosa*—A preliminary study. Regional Industries Research and Development Corporation, Australia, 2008.
- [5]. Anilreddy B: Preparation, characterization and biological evaluation of some overview of *Dodonaea viscosa* Linn. *J Pharmaceut Sci Tech* 2009;1:1–9.
- [6]. Rani MS, Rao, SP, Mohan K. *Dodonaea viscosa* Linn—an overview. *J Pharmaceut Res Health Care* 2009;1:97–112.
- [7]. Khalil MN, Sperotto SJ, Manfron PM: Anti-inflammatory activity and acute toxicity of *Dodonaea viscosa*. *Fitoterapia* 2006;77:478 – 480.
- [8]. Meenu J, Sunil S, Manoj K. Evaluation of antihyperglycemic activity of *Dodonaea viscosa* leaves in normal and STZ-diabetic rats. *Int J Pharm Pharmaceut Sci* 2011;3:69-74.
- [9]. Veerapur VP, Prabhakar KR, Parihar VK, Bansal P, Srinivasan KK, Priyadarsini KI, Unnikrishnan MK: Antidiabetic, hypolipidaemic and antioxidant activity of *Dodonaea viscosa* aerial parts in streptozotocin-induced diabetic rats. *Int J Phytomed* 2010;2:59–70.
- [10]. Kanter M, Coskun O, Budancamanak M. Hepatoprotective effects of *Nigella sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. *World J Gastroenterol* 2005;11:6684–6688.
- [11]. Nevin KG, Vijayamma L. Effect of *Aerva lanata* against hepatotoxicity of carbon tetrachloride in rats. *Environ Toxicol Pharmacol* 2005;20:471-477.
- [12]. Shankar GNL, Manavalan R, Venkappayya D, Raj CD. Hepatoprotective and antioxidant effects of *Commiphora berryi* (Arn) Engl bark extract against CCl₄-induced oxidative damage in rats. *Food Chem Toxicol* 2008;46:3182–3185.
- [13]. Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S. Effect of HD-03, an herbal formulation, on the antioxidant defense system in rats. *Phytother Res* 1998;12:114–117.
- [14]. Abu-Gabal NS, Abd-Alla HI, Ahmed HH, Al-Saigh SM, Shalaby NMM. Efficacy of Aloe vera extracts in attenuating neurological endangerment-induced by dexamethasone in adult male rats. *J Arab Soc Med Res* 2007;2:59-73.
- [15]. Wyk VEB. A broad review of commercially important southern African medicinal plants. *J Ethnopharmacol* 2008;119:342–355.
- [16]. Agrawal PK, Bansal MC. Flavonoid glycosides. In: studies in organic chemistry 39, ¹³C-NMR of flavonoids, Agrawal PK (Ed.), Elsevier science, New York, U.S.A., 1989.
- [17]. Mabry TJ, Markham KR, Thomas MB. The systematic identification of flavonoids. Springer-Verlag, Berlin, 1970;pp. 41–164.
- [18]. Markham KR. Techniques of flavonoid identification. Academic Press Inc, Ltd, London, 1982;pp. 84.
- [19]. Harborne JB, Mabry TJ. The Flavonoid: Advances in research, Chapman and Hall Ltd, London, 1982;pp. 1-18.
- [20]. Chen HY, Lin YC, Hsieh CL. Evaluation of antioxidants activity of aqueous extract of some selected nutraceutical herbs. *Food Chem* 2007;104:1418-1424.
- [21]. Marsillach J, Camps J, Ferré N, Beltran R, Rul A, Mackness B, Michael MM, Joven J. Paraoxonase-1 is related to inflammation, fibrosis and PPAR delta in experimental liver disease. *BMC Gastroenterol* 2009;9:3.
- [22]. Yuvaraj P, Subramoniam A. Hepatoprotective property of *Thespesia populnea* against carbon tetrachloride induced liver damage in rats. *J Basic Clin Physiol Pharmacol* 2009;20:169-177.
- [23]. Buege JA, Aust SD. Microsomal lipid peroxidation. *Meth Enzymol* 1978;52:302-310.
- [24]. Moron MS, Depierre JW, Mannervik B. Level of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. *Biochimica Biophysica Acta* 1979;582:67-78.
- [25]. Nishikimi M, Rae NA, Yagi K. The occurrence of superoxide anion in the action of reduced phenazine methosulphate and molecular oxygen. *Biochem Biophys Res Commun* 1972;46:849-853.
- [26]. Gella FJ, Olivella T, Cruz PM, Arenas J, Moreno R, Durban R, Gomez JA. A simple procedure for routine

- determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clin Chem Acta 1985;153:241-247.
- [27]. Rosalki SB, Foo AY, Burlina A. Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. Clin Chem 1993;39:648-652.
- [28]. Szasz G. A kinetic photometric method for serum γ -glutamyl transpeptidase. Clin Chem 1969;15:124-136.
- [29]. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-254.
- [30]. Suzuki H, Suzuki K. Rat hypoplastic kidney (hpk/hpk) induces renal anemia, hyperparathyroidism, and osteodystrophy at the end stage of renal failure. J Vet Med Sci 1998;60:1051-1058.
- [31]. Leenhouts PW. Notes on the extra-Australian species of *Dodonaea* (Sapindaceae). Blumea 1983;28:271-289.
- [32]. Horie T, Ohtsurs Y, Shibata K, Yamashita K, Tsukayama M, Kawamura Y. ^{13}C NMR special assignment of the a-ring of polyoxygenated flavones. Phytochemistry 1998;47:865-874.
- [33]. Rashid MA, Armstrong JA, Gray AI, Waterman PG. Alkaloids, flavonoids and coumarins from *Drummondita hassellii* and *D. calida*. Phytochemistry 1992;31:1265-1269.
- [34]. Salah NAM, Mansour RMA, Markham KR. An acylated isorhamnetin glycoside from *Aerva javanica*. Phytochemistry 1990;29:1344-1345.
- [35]. Halim AF, Saad HEA, Hashish NE. Flavonol glycosides from *Nitraria retusa*. Phytochemistry 1995;40:349-351.
- [36]. Sachdev K, Kulshreshtha DK. Flavonoids from *Dodonaea viscosa*. Phytochemistry 1983;22:1253-1256.
- [37]. Getie MG, Gebre-Miriam T, Rietz R, Neubert R. Distribution of quercetin, kaempferol and isorhamnetin in some Ethiopian medicinal plants used for treatment of dermatological disorders. Ethiopian Pharmacol J 2000;18:25-34.
- [38]. Abdel-Mogib M, Basaif SA, Asiri AM, Sobahi TR, Batterjee SM. New clerodane diterpenoid and flavonol-3-methyl ethers from *Dodonaea viscosa*. Pharmazie 2001;56:830-831.
- [39]. Fukumoto LR, Mazz G. Assessing antioxidant and prooxidant activities of phenolic compounds. J Agric Food Chem 2000;48:3597-3604.
- [40]. Jeong JM, Choi CH, Kang SK, Lee IH, Lee JY, Jung H. Antioxidant and chemosensitizing effects of flavonoids with hydroxy and/or methoxy groups and structure-activity relationship. J Pharm Pharm Sci 2007;10:537-546.
- [41]. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996;20:933-956.
- [42]. Saskia ABE, Acker V, Van-Den Berg DJ, Bast A. Structural aspects of antioxidant activity of flavonoids. Free Radic Biol Med 1996;20:331-342.
- [43]. Teffo LS, Aderogba MA, Eloff JN. Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. var. *angustifolia* leaf extracts. S Afr J Bot 2010;76:25-29.
- [44]. Manna P, Sinha M, Sil PC. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. BMC Comp Alt Med 2006;6:33-42.
- [45]. April JE, Michael PR, Gwendolyn LK, Edward JN, Evan BS. Experimental diabetes attenuates cerebral cortical - evoked forelimb motor responses. Diabetes 2005;54:2764-2771.
- [46]. Gharib B, Abd-Allah OM, Dessein H, De-Reggi M. Development of eosinophil peroxidase activity and concomitant alteration of antioxidant defenses in the liver of mice infected with *Schistosoma mansoni*. J Hepatol 1999;30:594-602.
- [47]. Mansour HH, Hafez HF, Fahmy, NM. Silymarin modulates cisplatin-induced oxidative stress and hepatotoxicity in rats. J Biochem Mol Biol 2006;39:656-661.
- [48]. Yadav NP, Pal A, Shanker K, Bawankule DU, Gupta AK, Darokar MP, Khanuja SPS. Synergistic effect of silymarin and standardized extract of *Phyllanthus amarus* against CCl_4 -induced hepatotoxicity in *Rattus norvegicus*. Phytomed 2008;15:1053-1061.
- [49]. Opoku AR, Ndlovu IM, Terblanche SE, Hutchings AH. In vivo hepatoprotective effects of *Rhoicissus tridentata* subsp. *cuneifolia*, a traditional Zulu medicinal plant, against CCl_4 -induced acute liver injury in rats. South African J Botany 2007;73:372-377.
- [50]. Romero FJ, Bosch-Morell F, Romero MJ, Jare o EJ, Romero B, Mar n N, Romá J. Lipid peroxidation products and antioxidants in human disease. Environ Health Perspectives 1998;106:1229-1234.
- [51]. Sharma N, Shukla S. Hepatoprotective potential of aqueous extract of *Butea monosperma* against CCl_4 induced damage in rats. Exp Toxicol Pathol 2011;63:671-676.
- [52]. Reyes-Gordillo K, Segovia J, Shibayama M, Vergara P, Moreno MG, Muriel P. Curcumin protects against acute liver damage in the rat by inhibiting NF-kappa B, proinflammatory cytokines production and oxidative

stress. *Biochim Biophys Acta* 2007;1770:989-996.

[53]. Hamed MA. Metabolic profile of rats after one hour of intoxication with a single oral dose of ethanol. *J Pharmacol Toxicol* 2011;6:158-165.

[54]. Wagner H. Trends and challenge: phytomedicine: research in the new millennium. In: Yaniv Z, Bachrach U (Eds.), *Handbook of Medicinal Plants*. Haworth press, New York, 2005;pp. 3-25.

[55]. Sivanesan D, Selvi VAVT, Bhakayaraj R, Arunachalam T. Protective effect of *Dodonaea viscosa* (L) against lead acetate induced altered glycoprotein profiles in rats. *E J Chem* 2009;6:725-728.

