NOVEL QUERCETIN GLYCOSIDE WITH PROMISING HEPATOPROTECTIVE ACTIVITY ISOLATED FROM *LOBULARIA LIBYCA* (VIV). C.F.W. (BRASSICACEAE)

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Abstract

A novel flavonoid compound characterized as Quercetin 3-O-α-L-rhamnopyranosyl (1'''→4'')-β-D-galactopyranosyl-7-O-α-L-rhamnopyranoside was isolated from the n-butanol fraction obtained from the ethanolic extract of *Lobularia libyca* (viv). C.F.W. (Brassicaceae). Also, two known flavonoids characterized as quercetin and kaempferol were isolated from the ethyl acetate fraction obtained from the ethanolic extract of the plant. The structure of the novel compound was established on the basis of extensive spectral and chemical studies. The complete spectral assignments of the isolated compounds are reported here on the basis of UV spectral analysis, 1D (^{1}H and ^{13}C) and 2D (HMBC) NMR and high resolution mass (HRMS) spectroscopic data. The results show that the n-butanol fraction has a promising antioxidant activity measured by 1,1-dipheny1-2-picrylhydrazine (DPPH) method with IC_{50} = 10.49 µg/ml. Moreover, the hepatoprotective activity of the n-butanol fraction as well as the new compound was studied using *in-vitro* technique by using human liver cell lines and measuring specific enzymes which are alanine transaminase (ALT), aspartate transaminase (AST), glutathione (GSH) and superoxide dismutase (SOD). The best activity was observed at

concentration 0.1 mg/ml of the n-butanol fraction. The fraction as well as the novel compound exhibit a promising hepatoprective activity comparable to a reference standard silymarin.

Keywords: *Lobularia libyca*, flavonoids, antioxidant, hepatoprotective

Introduction

Brassicaceae is one of the largest angiosperm families, the members of which are mostly concentrated in the temperate region and reach maximal diversity around the Mediterranean area. There is much diversity of opinion regarding the number of genera and species included in the family Brassicaceae throughout the world; they range from 338 to 380 genera and from 2500 to 3700 species (Willis, 1966, Cronquist, 1968, Al-Shehbaz, 1984, Al-Shehbaz, 1985, Lawrence, 1989, Heywwod, 1993, Al-Shehbaz *et al.*, 2006).

In the flora of Egypt, Brassicaceae is the fourth of eleven large families, widely distributed in all phytogeographic regions (Boulos, 1995). Different authors have disagreed on the number of genera and species recorded in Egypt; they vary from 38 to 55 genera and from 86 to 108 species (Montasir and Hassib, 1956, Tackholm, 1974, El-Hadidi *et al.*, 1988, El-Hadidi and Fayed, 1994/95, Boulos, 1999).

The genus *Lobularia* is represented by three species that grow in Egypt; *Lobularia maritima, L. arabica* and *L. libyca.* (Tackholm, 1974, Boulos, 1999).

Many species within the Brassicaceae have been surveyed for their flavonoid profiles. Four different kaempferol glycosides were isolated from the other species of the genus *Lobularia* which is *L.maritima* (sweet alyssum) (Fiorentino *et al.*, 2009). Many systematic studies of the flavonoids in the genera *Brassica* and *Sinapis* were carried out (Durkee and Harborne, 1973, Nielsen *et al.*, 1993). They showed that flavonol glycosides were the only flavonoids present in the leaves and flowers of these two species. Flavonol glycosides were also found in many other members of the Brassicaceae (Rizk, 1986, Sanchez-Yelamo and Martinez-Laborde, 1991, Aguinagalde *et al.*, 1992, Nielsen *et al.*, 1993, Nielsen *et al.*, 1998, Werkerle *et al.*, 2001) . Moreover, Sheahan and Cheong (1998) reported the accumulation of flavonols in *Arabidopsis thialana*. Several studies showed the overall amount of soluble flavonols (quercetin and kaempferol glycosides) in leaves of *Brassica napus* increased substantially after exposure to supplementary UV-B radiation (Greenberg *et al.*, 1996, Olsson *et al.*, 1998, Wilson *et al.*, 1998). In addition to flavonol derivatives, Onyilagha *et al.* (2003) reported derivatives of the flavones, apigenin and luteolin, in species of *Crambe* and *Thlaspi*. Forkmann (1979) reported

flavanones and dihydroflavonols in species of *Matthiola.* Finally, to the best of our knowledge there is no phytochemical or biological studies were carried out on *L.libyca,* so this is the first work concerning this plant.

Material and methods

Plant material

Fresh herbs of *Lobularia libyca* (viv) C.F.W. Meissn. (Brassicaceae) were collected in March (2011) from the northern coast (Alexandria-Marsa Matrouh Road) and identified by Prof. Abdel-Halim Abdel-Mogaly, Department of Plant Taxonomy, Herbarium of Horticultural Research Institute, Agricultural Research Centre , Dokki, Cairo, Egypt. The plant was identified according to Tackholm (1974) and Boulos (1999). The collected plant was visually matched against herbarium specimens at the herbarium of the Horticultural Research Institute, Dokki and a voucher specimens was deposited there.

Instruments

Ultra-Violet spectra were recorded on UV-visible spectrophotometer (Shimadzu model 2401 PC). NMR measurements were carried out using Jeol EX-500 spectroscopy; 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) and Finnigan LTQ FT Ultra mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a Nanomate ESI interface (Advion).

Extraction and fractionation

The fresh plant (2 kg) was refluxed with 70% ethanol for 15 min. in order to deactivate the plant enzymes. The plant was dried and comminuted to powder, extracted by maceration in 70% ethanol on cold and filtered. The process was repeated until complete exhaustion and the combined filtrates were concentrated under reduced pressure at 45^{o} C to produce 105 g of a sticky dark brown residue. The alcoholic extract was further fractionated using solvents of increasing polarities beginning with petroleum ether followed by methylene chloride, ethyl acetate and finally n-butanol. Each fraction was dried over anhydrous sodium sulfate and concentrated under vacuum to afford petroleum ether (6 g), methylene chloride (2 g), ethyl acetate (1 g) and n-butanol (19 g) fractions.

Total phenolics content (Folin-Ciocalteu method)

The phenolic compounds were determined using the Folin-Ciocalteu method, based on the reduction of phosphor-wolframate-phosphomolybdate complex by phenolics to a blue reaction product (Singleton and Rossi, 1965, Bonoli *et al.*, 2004, Kyoung *et al.*, 2005). The Folin-Ciocalteu reagent was diluted 10 times (2.5 ml) and mixed with 2 ml of saturated sodium carbonate (75 g/L) and 50 µl of sample (total alcoholic extract, ethyl acetate and n-butanol fractions, each one separately) and shaken for 10 s and heated for 30 min at 45°C. The absorbance was measured at 765 nm after cooling at room temperature. Total phenolics content were calculated as µmol gallic acid

equivalents per gram of fresh plant from a calibration curve prepared by gallic acid solutions at concentrations 212-1062 μmol /L.

Total flavonoids content

Colorimetric aluminum chloride method was used for flavonoids determination that was modified from the method reported by Woisky and Salatino (1998) and Nabavi *et al.* (2008). Briefly, 0.1 g of each sample (total alcoholic extract, ethyl acetate and n-butanol fractions) was mixed with 25 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. Total flavonoids content were calculated as quercetin equivalents from a calibration curve prepared by quercetin solutions at concentrations 12.5 to 150 mg/ ml in methanol.

Isolation of flavonoids

Compounds 1 and 2 were isolated from the ethyl acetate fraction using preparative paper chromatography (PPC) (Whatman no.3MM) and solvent system n-butanol : acetic acid : water (BAW) with a ratio 4:1:5 by descending technique. The compounds were identified using ^{1}H and ^{13}C-NMR and UV-spectral analysis and confirmed by co-elution with standard quercetin and kaempferol flavonoids. The n-butanol fraction (15 g) was subjected to a polyamide (Sigma-Aldrich, Germany) column (100 × 5 cm) starting with water as eluent then decreasing the polarity by increasing the percentage of methanol. fourty fractions were collected each of about 500 ml. Similar fractions were combined according to their paper chromatography (PC, Whatman no.1) properties using H_2O, 15% Acetic acid and BAW as eluents to give 6 main fractions, Compound 3 was isolated from fraction 2 using PPC and BAW as a mobile phase. The rest of the fractions contain minor undifferentiated spots.

Enzyme hydrolysis of compound 3

The enzyme hydrolysis of compound 3 was carried out according to the method described by Wang *et al.* (2012). The hesperidinase enzyme from *Aspergillus niger* was supplied from Sigma-Aldrich, Germany. 10 mg hesperidinase enzyme was mixed with 20 mL 2 g/L aqueous solution of compound 3 and diluted by another 20 ml water at pH = 6. The mixture was incubated at 40^{o} C for 30 min. The hydrolysate was purified by extraction with ethyl acetate followed by concentration under vacuum at 45^{o} C yielding finally 31 mg of the extract that was applied on silica gel column chromatography (50 × 1 cm) with n-hexane – ethyl acetate (1:1, v/v) as mobile phase, and crystallized in mixed solvent methanol–water (7:3, v/v).

Antioxidant assay by DPPH method

The DPPH radical scavenging assay was first described by Blois (1958) and was later modified by numerous researchers. This method is

based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolorizes the DPPH solution. The antioxidant activity is measured by the decrease in the absorbance at 517 nm. 2 ml of 0.1 mM solution of DPPH in methanol was added to 2 ml of the serial dilutions of the samples (ethyl acetate and n-butanol fractions each one separately) at concentrations of 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.5, 0.7 and 1 mg/ml. L-Ascorbic acid was used as standard at concentrations 0.25, 0.5, 0.75, 1, 2, 4, 6 and 8 µg/ml. After incubation for 30 min at room temperature, the absorbance was measured. This activity is expressed as percentage DPPH radical-scavenging that is calculated according to the following equation:

$$\% \text{ DPPH radical-scavenging} = (A_C - A_S)/AC \times 100$$

Where A_C is the absorbance of the control solution (containing only DPPH in methanol), A_S is the absorbance of sample in DPPH solution. The percentage of DPPH radical-scavenging was plotted against the plant extract concentrations (mg/ml) to determine the concentrartion of extract required to scavenge DPPH by 50% (IC_{50}). (Ratty *et al.*, 1988, Miliauskas *et al.*, 2004)

Assay of hepatoprotective activity

a- Cell Culture

Hep-G2 cell line was purchased from VACSERA, Giza, Egypt, and maintained in tissue culture unit, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. Cells were grown in Dulbecco's modified Eagle's medium supplemented with heat-inactivated fetal bovine serum (10%), penicillin G (100 IU/ml), and streptomycin (100 µg/ml). Cells were maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity. Cell culture reagents were obtained from Lonza (Basel, Switzerland) (Torres-Gonzales *et al.*, 2011).

b- *In-vitro* assay of heptoprotective effect

The hepatoprotective activity of the n-butanol fraction and compound 3 were tested *in vitro* at three different concentrations (1, 0.1 and 0.01 mg/ml) and compared to the standard hepatoprotective agent silymarin (Sil) at the same concentrations. Hep-G2 monolayer culture after attachment was pretreated with the hydroalcoholic solution of each sample or silymarin for one hour. An aliquot of 40 mM CCl_4 in 0.05% dimethyl sulfoxide (DMSO) was added and incubation was continued for another two hours. The supernatant medium and cell lysate were collected and stored at -20 °C until analysis. Positive control was a set of cells maintained in culture medium and treated only with CCl_4 (40 mM); while the negative control was a set of cells maintained in phosphate-buffered saline. The levels of ALT, AST, GSH & SOD activity were assessed in the supernatant using commercially available kits according to the manufacturer instructions (Biodiagnostics, Cairo, Egypt) (Torres-Gonzales *et al.*, 2011).

Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by analysis of variance ($p<0.05$) and the means separated by Duncan's multiple range tests (by InStat3 software). The IC_{50} values were calculated from linear regression analysis.

Results and discussion

Total phenolics and flavonoids contents

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well-being. Different extracts of *Lobularia libyca* (viv) C.F.W. Meissn. were analyzed for their phytocnostituents. Total phenolic compounds, as determined by Folin-Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y = 0.0053x$, $r2 = 0.987$). The total flavonoids content is reported as mg quercetin equivalent/g of fresh plant, by reference to standard curve ($y = 0.0028x – 0.0001$, $r2 = 0.9947$). The quantitative estimation of the different extracts and fractions shows that the plant is rich in total phenolics and total flavonoids according to the data shown in table 1. The results show that the total alcoholic extract has the highest amount of phenolics and flavonoids. Moreover, n-butanol fraction content of phenolics and flavonoids is much higher than the ethyl acetate fraction content that may indicate the higher antioxidant activity of the n-butanol fraction than the ethyl acetate one. It is well known that the plant flavonoids and phenols in general, are highly effective free radical scavenging and antioxidants. Polyphenol and flavonoids are used for the prevention and cure of various diseases which are mainly associated with free radicals (Havesteen, 1983, Shahidi and Wanasundara, 1992, Deepa *et al.*, 2009).

Table (1) Total phenolics and flavonoids contents of different extracts from *Lobularia libyca* (viv) C.F.W.

Plant extract	**Total phenolics (mg GAE/g fresh plant)**	**Total flavonoids (mg quercetin/g fresh plant)**
Total alcoholic Ext.	25.261 ± 0.031	3.56 ± 0.102
Ethyl acetate fraction	0.98 ± 0.012	0.099 ± 0.016
n-Butanol fraction	8.231 ± 0.092	1.05 ± 0.058

Identification of the isolated compounds

Flavonoids were identified by chromatographic analysis of enzyme hydrolysates and by spectroscopic methods. The UV-spectra of flavonoids were recorded in MeOH(a), also after addition of NaOMe(b), $AlCl_3$(c), $AlCl_3$/HCl(d), NaOAc(e) and NaOAc/H_3BO_3(f), according to Marby *et al.* (1970)

Compound 1 (Kaempferol)

$C_{15}H_{10}O_6$, yellow needles (24 mg), yellow fluorescence under UV light, R_f = 0.1 (6% acetic acid), 0.85 (BAW), mol.wt. 286; negative ion ESI-MS m/z 285 $[M-H]^-$; UV (at λ_{max}, nm) (a) 268, 369; (b) 278, 316, 413; (c) 270, 305, 360, 430; (d) 255, 269, 348, 422; (e) 270, 310, 375; (f) 270, 320, 372. ^{1}H NMR (DMSO, 500 MHz, δ = ppm): 6.18 (1H, *d, J* = 2.5 Hz, H-6), 6.4 (1H, *d, J* = 2.5 Hz, H-8), 6.89 (2H, *d, J* = 8 Hz, H-3' and H-5'), 8.14 (2H, *d, J* = 8 Hz, H-2' and H-6'). ^{13}C NMR (DMSO, 125 MHz): (Table 2).

Compound 2 (Quercetin)

$C_{15}H_{10}O_7$, yellow needles (35 mg), yellow fluorescence under UV light, R_f = 0.13 (6% acetic acid), 0.75 (BAW), mol.wt. 302.3; negative ion ESI-MS m/z 301 $[M-H]^-$; UV (at λ_{max}, nm) (a) 255, 268, 370; (b) 247, 321; (c) 270, 360, 440; (d) 258, 400; (e) 254, 276, 375; (f) 272, 388. ^{1}H NMR (DMSO, 500 MHz, δ = ppm): 6.18 (1H, *d, J* = 2.0 Hz, H-6), 6.39 (1H, *d, J* = 2.0 Hz, H-8), 6.89 (1H, *d, J* = 8.0 Hz, H-5'), 7.63 (1H, *dd, J* = 2.0, 7.5 Hz, H-6'), 7.72 (1H, *d, J* = 2.0 Hz, H-2'), ^{13}C NMR (DMSO, 125 MHz): (Table 2).

Compound 3 (Quercetin 3-O-α-L-rhamnopyranosyl (1'''→4'')-β-D-galactopyranosyl-7-O-α-L-rhamnopyranoside) (figure 1)

$C_{33}H_{40}O_{20}$, dark yellow needles (73 mg), dark purple fluorescence under UV light, Rf = 0.83 (6% acetic acid), 0.83 (BAW), mol.wt. 756; negative ion ESI-MS m/z 755 $[M-H]^-$; UV (at λ_{max}, nm) (a) 256, 358; (b) 270, 396; (c) 275, 441; (d) 270, (295), (365), 402; (e) 262, 412; (f) 261, 380. ^{1}H NMR (DMSO, 500 MHz, δ = ppm): 0.73 (3H, d, J = 6.1 Hz, H-6"'); 1.08 (3H, d, J = 6.1 Hz, H-6""); 5.04 (1H, J = 1.8 Hz, H-1"'); 5.51 (1H, J = 1.8 Hz , H-1""); 5.61 (1H, d, J = 7.6 Hz, H-1"); 6.37 (1H, d, J = 2.0 Hz, H-6), 6.74 (1H, d, J = 2.0 Hz ,H-8), 6.78 (1H, d, J = 8.4 Hz, H-5'); 7.52 (1H, d, J = 1.55 Hz, H-2'); 7.70 (1H, dd, J = 2.5, 8.5 Hz, H-6'), ^{13}C NMR (DMSO, 125 MHz): (Table 2).

Spectral data of compound 3 after hydrolysis by hesperidinase enzyme

^{1}H NMR (DMSO, 500 MHz, δ = ppm): 5.37 (1H, d, J = 7.6 Hz, H-1"); 6.24 (1H, d, J = 2.0 Hz, H-6); 6.47 (1H, d, J = 2.0 Hz, H-2); 6.88 (1H, d, J = 8.4 Hz, H-5'); 7.60 (1H, d, J = 1.55 Hz, H-2'); 7.69 (1H, dd, J = 2.5, 8.5 Hz, H-6'), ^{13}C NMR (DMSO,125 MHz): (Table 2).

The ^{1}H-NMR, ^{13}C-NMR and UV spectral data of compounds 1 & 2 were found to be in accordance with the proposed structures of kaempferol & quercetin. Also, comparison with authentic samples flavonoids using PC confirmed the identity of compounds 1 & 2 to be kaempferol & quercetin respectively (Marby *et al.*, 1970, Harborne, 1982, Markham, 1982).

The ^{1}H-NMR of compound 3 shows a typical pattern of quercetin aglycone (Han *et al.*, 2001), chemical shifts of H-C6 and H-C8 at δ 6.37 & 6.74 ppm respectively are downfield than their normal positions indicating that there is a substitution at C7 (Iwashina *et al.*, 1995, Han *et al.*, 2001), that was confirmed by the UV spectra which indicated that the hydroxyl groups attached to C3 and C7 are occupied by a substitution while the hydroxyl groups attached to C-3' and C-4' are free (Marby *et al.*, 1970, Markham, 1982). Also there are 3 duplets at δ 5.04, 5.51 & 5.61 ppm of the anomeric protons of three sugars, that were found to be two L-rhamnosyl moieties and one D-galactosyl moiety, the two rhamnosyl moieties were evidenced by presence of two duplets with integration of three protons at δ 0.73 & 1.08 ppm of H-C6'" and H-C6"" respectively (Kang *et al.*, 1988). The anomeric protons had a coupling constants of 1.8 Hz in case of rhamnose confirming its α orientation and 7.6 Hz in case of galactose indicating diaxial coupling of the β pyranosyl configuration. The anomeric of one rhamnosyl moiety is downfield than the another one indicating that one is directly attached to the aglycone while the second one is attached to a sugar, this was confirmed by the 2D-HMBC spectra which is described later and the hydrolysis by hesperidinase enzyme yielding quercetin 3-O-β-D-galactoside and α-L-rhamnose identified by comparing their H1 and C13 NMR data with literature (Markham *et al.*, 1976).

The ^{13}C-NMR confirmed the results obtained from ^{1}H-NMR, the difference between C-3" at δ 74.63 ppm and C-5'' at δ 76.27 ppm is more than 1 ppm (exactly 1.74 ppm) confirming that it is a galactosyl not a glucosyl moiety (Kang *et al.*, 1988), Also, there as a significant downfield of C-4" at 75.73 ppm with slight upfield of C3" and C4" suggesting that linkage between the two sugars is (1→4) (Lepri *et al.*, 1987).

The HMBC analysis confirmed the linkages of the sugars to the aglycone and the linkage between the two sugars. There is a cross peak between C-7 at δ 162.05 ppm and H-1"" at δ 5.51 ppm confirming that there is an α-L-rhamnosyl moiety at C-7, also there is another cross peak between C-3 at δ 133.67 ppm and H-1" at δ 5.61 ppm confirming that the β-D-galactosyl moiety is directly attached to OH of position 3. For confirming the glycosidic linkage between the two sugars; there are cross peaks between H-1"'at δ 5.04 ppm and C-5"' at δ 68.67 ppm, C-2"' at 71.18 ppm and C-4" at δ 75.73 ppm, the C-4'' is confirmed that it have cross peaks with doublet proton at δ 3.55 ppm of H-6" a, doublet proton at δ 3.45 ppm of H-6" b, Multiplet proton at δ 3.4 ppm of H-5", Triplet proton at δ 3.26 ppm of H-3" and finally with H-1"' at δ 5.04 ppm. To the best of our knowledge this compound is novel and this is the first report of isolation and identification of flavonoids of *Lobularia libyca* (viv.) C.F.W.

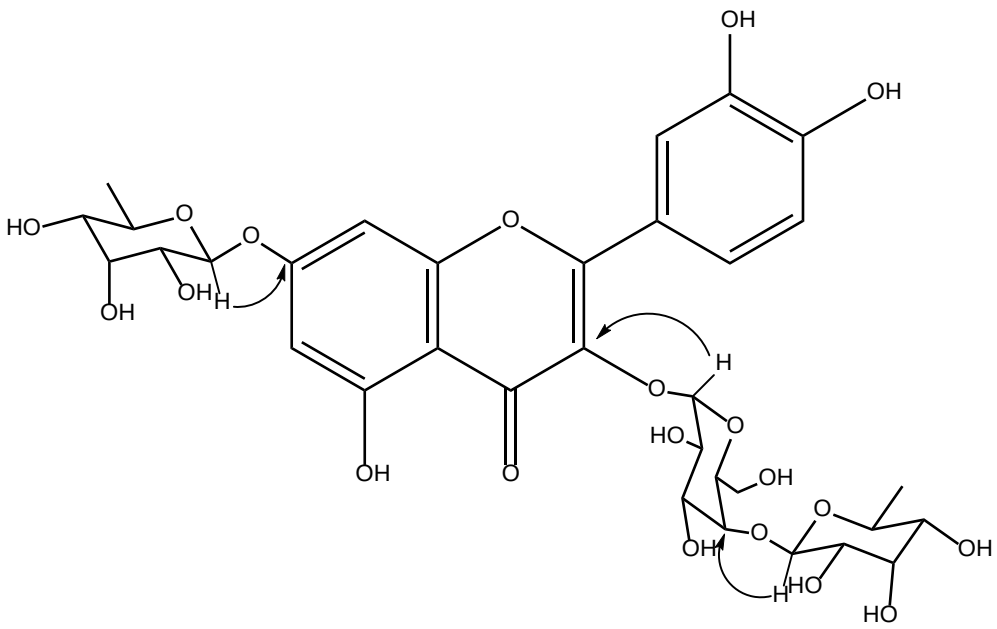

Fig. (1) Compound 3: Quercetin 3-O-α-L-rhamnopyranosyl (1’’’→4’’)-β-D-galactopyranosyl-7-O-α-L-rhamnopyranoside showing significant correlation in HMBC spectra.

DPPH radical scavenging activity

The ethyl acetate and n-butanol fractions were subjected to screening for their possible antioxidant activities. DPPH, a stable free radical with a characteristic absorbance at 517 nm, was used to study the radical scavenging effects of the fractions. As antioxidants donate protons to these radicals, the absorbance decreases which is taken as a measure of the radical scavenging. The results of free radical scavenging capacities of the fraction accompanied by ascorbic acid as a standard were shown in table 3.

The results represented by IC_{50} which is the concentration that inhibits 50% of the used DPPH amount. It was determined by correlation between the sample concentration and the free radical inhibition capacity IC (%). The results show that the antioxidant activity of the n-butanol fraction (IC_{50} = 10.49 µg/ml) is much more than the activity of the ethyl acetate fraction (IC_{50} = 140 µg/ml). The results of the antioxidant activities were well correlated with the results of the total phenolics and flavonoids as that the n-butanol fraction contains more phenolics and flavonoids than the ethyl acetate fraction so its antioxidant activity is much more higher that the ethyl acetate fraction. Because of the high antioxidant activity of the n-butanol fraction, it is further subjected to investigate its hepatoprotective activity.

Table (2) ^{13}C-NMR (DMSO, 125 MHz, δ = ppm) data of compounds 1-3

Carbon no.	**Compound 1**	**Compound 2**	**Compound 3**	**Compound 3 after enzyme hydrolysis**
Aglycone				
2	146.1	147.9	157.15	156.3
3	135.5	137.2	133.67	133.60
4	175.7	177.3	177.89	177.42
5	156.0	162.5	161.40	161.12
6	98.2	99.3	99.33	98.64
7	163.8	165.7	162.05	164.05
8	93.4	94.4	94.76	93.96
9	160.5	158.2	156.33	156.33
10	102.9	104.4	106.08	105.78
1’	121.6	124.1	121.05	121.15
2’	129.3	116.0	115.82	115.92
3’	115.3	146.3	145.69	144.89
4’	159.0	148.7	149.79	149.45
5’	115.3	116.2	116.27	116.07
6’	129.3	121.6	122.72	121.92
Galactose				
1”			101.06	102.6
2”			71.18	71.15
3”			74.63	75.15
4”			75.73	67.2
5”			76.27	77.17
6”			60.6	60.23
Rhamnose 1				
1’”			99.93	
2’”			71.18	
3’”			70.32	
4’”			72.44	
5’”			68.67	
6’”			17.74	
Rhamnose 2				
1””			101.06	
2””			70.79	
3””			70.57	
4””			72.17	
5””			69.03	
6””			18.44	

Table (3) DPPH radical scavenging activity of different extracts from *Lobularia libyca* (viv) C.F.W.

Sample	**DPPH radical scavenging activity IC_{50} (µg/ml)**
Ethyl acetate fraction	140 ± 1.23
n-Butanol fraction	10.49 ± 0.21
L-ascorbic acid (Vitamin C)	1.26 ± 0.013

In-vitro assay of the hepatoprotective activity

The effects of the n-butanol fraction as well as the isolated compound **3** on Hep-G2 cells are shown in figure 2. AST and ALT levels were significantly elevated after treatment of cells with CCl_4. The treatment of cells with either the n-buanol fraction (1, 0.1 and 0.01 mg/ml) or compound **3** (1, 0.1 and 0.01 mg/ml) showed significant concentration-related amelioration of CCl_4-induced damage in cell membrane evidenced from normalized values of ALT and AST (Table 4 and figure 2) . The effect of the tested samples on the hepatocyte membrane integrity was comparable to that of the reference standard silymarin which is sometimes higher than the standard in case of the n-butanol fraction.

Pretreatment of Hep-G2 cells with the samples (1, 0.1 and 0.01 mg/ml) replenished the levels of GSH concentration and SOD activity significantly in a concentration-related manner, compared to cells treated with CCl_4 only. The reference standard drug silymarin also caused significant amelioration of the effects of CCl_4 on GSH and SOD levels but less than the amelioration caused by the two samples (Table 4 and figure 3).

Many plant-derived natural products worldwide have the potential to be protective and therefore can be used to treat acute and chronic liver diseases (Hamburger and Hostettmann, 1991, Luper, 1998, Luper, 1999, Scuppan *et al.*, 1999, Seeff *et al.*, 2001). The hepatoprotective effects of plants are mainly attributed to the presence of flavonoids, coumarins, phenolic acids (Pietta, 2000, Fogden and Neuberger, 2003, Ball and Kowdley, 2005, Mayer *et al.*, 2005). Also they may be attributed to their antoxidant activity because antioxidants scavenge free radicals in cases of liver disease (Shyamal *et al.*, 2006, Dandagi *et al.*, 2008). The heptoprotective effect of *Silybum marianum*, a member of the Asteraceae family, is mainly due to its antioxidant flavonoid content (Ball and Kowdley, 2005, Mayer *et al.*, 2005, Abenavoli *et al.*, 2010). Similarly, the promising hepatoprotective effect of *Lobularia libyca* is mainly attributed to the high flavonoid content and the powerful antioxidant properties of its extract. That reasons are evidenced by the highest activity of the n-butanol fraction which contains mainly flavonoid glycosides and also the activity of its major compound which is a novel quercetin glycoside.

To the best of our knowledge this is the first study to report the *in vitro* hepatoprotective effects of *Lobularia libyca* extracts using a cell culture model consisting of human hepatoma cell line that was treated with CCl_4 to induce hepatocyte damage.

Table (4) Results of *in-vitro* assay of hepatoprotective effect of *Lobularia libyca* (viv.) C.F.W.

Samples		**Enzyme activity (Unit/ml)**			**GSH level (mg/dL)**
		AST	**ALT**	**SOD**	**GSH**
Normal		17.74 ± 6.5	12.74 ± 1.96	385.71 ± 23.35	16.22 ± 0.39
CCl_4		124.16 ± 16.44	91.87 ± 3.4	50.00 ± 3.09	5.98 ± 0.56
n-Butanol fraction	**(1 mg/ml)**	15.86 ± 5.2	13.93 ± 0.03	279.32 ± 6.8	21.20 ± 0.07
	(0.1 mg/ml)	25.07 ± 0.36	19.14 ± 0.09	262.62 ± 4.22	20.12 ± 0.10
	(0.01 mg/ml)	40.04 ± 0.36	22.46 ± 0.11	187.03 ± 2.72	16.63 ± 0.01
Compound 3	**(1 mg/ml)**	32.54 ± 2.33	29.63 ± 3.54	257.14 ± 9.28	18.09 ± 0.5
	(0.1 mg/ml)	52.29 ± 0.88	42.62 ± 2.95	176.79 ± 16.07	16.93 ± 0.47
	(0.01 mg/ml)	64.49 ± 6.09	61.01 ± 4.39	94.64 ± 6.19	16.84 ± 0.38
Silymarin	**(1 mg/ml)**	21.32 ± 5.04	18.71 ± 4.16	308.93 ± 6.19	17.02 ± 0.37
	(0.1 mg/ml)	26.93 ± 0.49	32.16 ± 3.2	275.00 ± 13.48	16.69 ± 0.28
	(0.01 mg/ml)	45.79 ± 12.54	45.46 ± 3.1	212.5 ± 8.18	14.99 ± 0.27

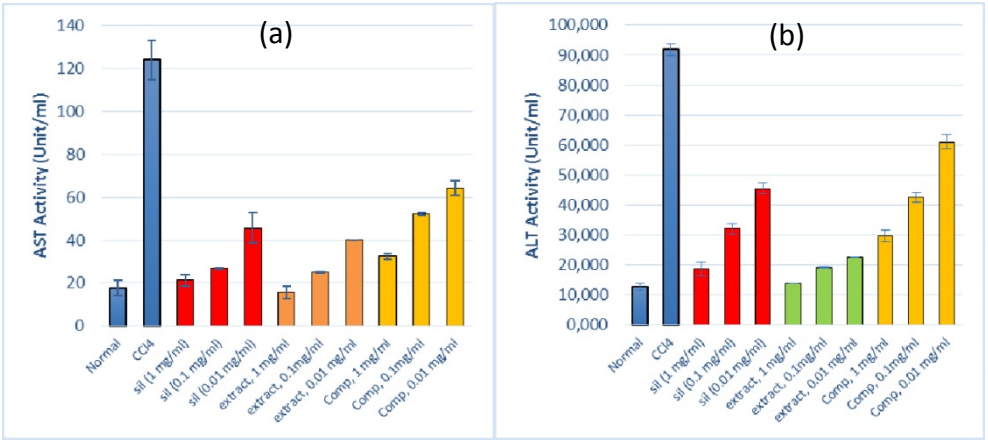

Fig. (2) Levels of AST and ALT enzymes before and after application of the n-butanol fraction, compound 3 and silymarin on damaged liver cells. (a) AST levels, (b) ALT levels

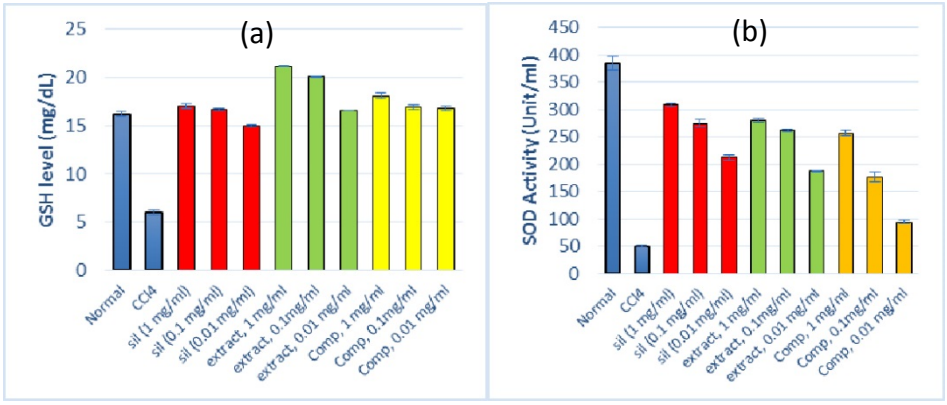

Fig. (3) Levels of GSH and SOD enzymes before and after application of the n-butanol fraction, compound 3 and silymarin on damaged liver cells. (a) GSH levels, (b) SOD levels.

Conclusion

Lobularia libyca (viv) C.F.W. (Brassicaceae) is a plant rich in phenolics and flavonoids with promising hepatoprotective activity. Three flavonoids isolated from *L. libyca*, one of them is a novel quercetin glycoside isolated and identified for the first time, further analysis for hepatoprotective effect including *in-vivo* analysis is recommended for confirming its activity inside a biological system.

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