Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

CHEMICAL AND BIOLOGICAL CONSTITUENTS FROM THE LEAF EXTRACTS OF THE WILD ARTICHOKE (CYNARA CORNIGERA)

[1]ELSAYED SM, [1]NAZIF NM, [1]HASSAN RA, [1]HASSANEIN HD, [2]ELKHOLY YM, [2]GOMAA NS, [1,3]SHAHAT AA

[1]Phytochemistry Department, National Research Centre, 12311 Dokki, Cairo, Egypt. [2]Departments of Chemistry, Helwan University, Cairo, Egypt, [3]Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia. Email: aashahat@hotmail.com

Received: 11 Sep, 2012, Revised and Accepted: 12 Oct, 2012

ABSTRACT

Five compounds have been recognized as cynaroside **1**, apigenin-7-O-glucoside **2** and scolymoside **3** and two sesquiterpens viz. grosheimin **4** and solstitalinA **5**. Their structures were elucidated by spectral methods. Compounds **4** and **5** were isolated from *Cynara cornigera* for the first time. The antioxidant potential was found to be (89.8%), for EtOAc fraction, (88.46%) for MeOH extract and (74.8%) for mother liquor fraction. Compounds **1** and **3** were isolated as the active principles from the EtOAc fraction with antioxidant activities 90.2, & 90.5% respectively using DPPH° assay comparing to the reference trolox (91.6 %). Compound **2** gave weak activity. No antioxidant activity was noticed for compounds **4** and **5**. The EtOAc fraction and the 80% methanolic extract exhibited hepatoprotective activity at a concentration of 12.5 µg/mL. Compounds **1** and 3 showed hepatoprotective activity at a concentration of 50 and 25 µg/mL, respectively on using the monolayer hepatocytes assay.

Keywords: *Cynara Cornigera*, Asteraceae, Flvonoids, Sesquiterpens, Hepatoprotective, antioxidant.

INTRODUCTION

Artichoke is an ancient herbaceous plant, belonging to family Asteraceae, originating from the Mediterranean area, which today is widely cultivated all over the world. Its flower head is eaten as a vegetable and prepared for different value-added products such as salad, jam, concentrate, and canned beverages[1]. Extracts from artichoke, *Cynara scolymus* L., have been used in folk medicine against liver complaints and the extracts or its constituents have been claimed to exert a beneficial action against hepato-biliary diseases [2, 3] and to improve liver regeneration after partial hepatectomy [4].

Several herbal products are available all over the world with an acclaimed hepatoprotective activity that is considered to be less toxic and free from side effects. The leaves of Artichoke are frequently used in folk medicine in many countries, to treat several ailments, including hepatitis, hyperlipidemia, and obesity, dyspeptic disorder, among others. Clinical and pre-clinical trials have confirmed the therapeutic potential of this plant, particularly in the treatment of hepato-biliary dysfunction and digestive complaints, and also its effectiveness in patients with irritable bowel syndrome and hyperlipoproteinemia, as well as its choleretic and antioxidant effects [5-7].

The therapeutical activity of the crude extract is probably first of all due to the phenolic structure of these substances that is responsible of the free radical mediated processes inhibition[8,9]. Free radical and lipid peroxidation are known to play an important role in a great number of pathological states [10-11].

Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders.

The objective of this study was to evaluate the *in vitro* hepatoprotective and antioxidant activities of the 80% of methanol extracts, different fractions and some of the isolated compounds from the leaves of *C. cornigera* growing wildly in Egypt.

In this report, we describe the isolation and structure elucidation of three flavonoids and two sesquiterpens, two of them i. e. compound 4 and 5 were isolated for the first time from this species. The identification of these compounds was undertaken by comparing the spectroscopic data of these compounds with those published earlier.

MATERIAL AND METHODS

General

Thin layer chromatography was carried out on precoated Silica gel F_{254} plates (Merck, Darmstadt, Germany) developed with Hexane:ethyl acetate (9:1) (solvent 1 for sesquiterpens), spots were detected using vanillin-H_2SO_4 (vanillin 1% in methanol and 5% H_2SO_4) followed by heating the plates to 110 and ethyl acetate–acetic acid–formic acid–water (30: 0.8: 1.2: 8) (solvent 2 for flavonoids). Spots were detected using Neu's spray reagent (1% diphenylboric acid ethanolamine complex in methanol). Column chromatography (CC) was performed using Silica gel (Merck) and Sephadex LH-20 (Merck) (Darmstadt, Germany). NMR was recorded on a Bruker DRX-400 spectrometer operating at 400 MHz for H and at 100 MHz for C. Chemical shifts are presented in ppm downfield of TMS.

Plant material

The aerial part of *Cynara Cornigera* (Family Asteraceae) was collected during flowering period (March 2009) from Marsa-Matroh, Egypt. Herb was dried in oven at 40 °C, finely powdered and kept for phytochemical and biological studies.

The plant was kindly authenticated by Prof. Dr. Ibrahim El-Garf, Botany Department, Faculty of Science, Cairo University, Cairo, Egypt.

Extraction, fractionation and isolation:

Five hundred gram air dried powdered herb of wild Artichoke (*cynara cornigera*) was extracted by maceration in aqueous methanol (80%) three times. The extracts were combined and evaporated under reduced pressure at 45°C to yield a dark brown residue 100 g. 20 g of this residue was subjected to silica gel column chromatography and eluted firstly with 100% hexane, followed by increasing amounts of methylene chloride in hexane as follows: methylene chloride (CH_2Cl_2): hexane (50:50), (75:25) and 100% CH_2Cl_2, then increase polarity with methanol in CH_2Cl_2 with an increment of 10% up-to methanol (100%). Two hundred fractions (100 ml each) were collected, and monitored on TLC silica gel plates. The plates were developed using solvent 1 or solvent 2 and sprayed with reagent A or reagent B. The spots were detected under UV before and after spraying. Fractions were combined together based on their TLC pattern in 7 subfrations A to G. The sesquiterpens were found in subfraction A (1 g) which eluted with EtOAc/Hexane (60:40) and flavonoid glycosides were found in subfraction C (2 g) & E (1.5 g). Further fractionation of subfraction A on silica gel column chromatography (90 g, 3x60 cm) using a hexane and increase polarity with EtOAc, compound **4** (42 mg) was eluted with solvent of hexane/EtOAc (40:60) and compound **5** (90 mg) was eluted with hexane/EtOAc (20: 80). Subfraction C was further purified by column chromatography over Sephadex LH-20 to give compound **1** (96 mg). Subfraction E was subjected to column chromatography

over Sephadex LH-20 and preparative thin layer chromatography using solvent system 2 to yield compounds **2** (12mg) and **3** (30 mg).

***Luteolin-7-O-glucoside (cynaroside)* (1)**

(96 mg) was obtained as yellowish brown particles with Rf = 0.4 (system 2). UV λ max in MeOH: 254, 349; NaOMe: 265, 390; (NaOAc) 257, 355; (NaOAc/H_3 BO_3) 261, 373; (AlCl3) 270, 389; ($AlCl_3$/HCl) 269, 360, 384. ^{1}H NMR (DMSO- d_6) d (ppm): 7.32 (d, 2H, H-2`, 6`), 6.86 (d, 1H, H-5`), 6.7 (d,1H, H-3,8), 6.40 (d,1H, H-6), and an anomeric proton of glucose at 4.95 (1H, H-1``), broad peak at 3.6-3.1 attributed to protons of sugar moiety.

***Apigenin-7-O-glucoside* (2)**

(8 mg) was obtained as dark brown particles with Rf = 0.5 (system 2). UV λ max MeOH: 286, 333; (NaOH) 274, 391; (NaOAc) 268, 358, 388; (NaOAc/H3 BO3); 267, 339; (AlCl3) 272, 300, 337; ($AlCl_3$ /HCl) 273, 299, 340.

***Luteolin-7-O-rhamnoglucoside (scolymoside)* (3)**

(50 mg) was obtained as dark brown particles with Rf = 0.14 (system 2). UV λ max MeOH: 255, 346, 350; (NaOH) 265, 317, 399; (NaOAc) 241, 256, 354; (NaOAc/H_3 BO_3); 262, 377; ($AlCl_3$) 273, 332, 431; ($AlCl_3$ /HCl) 274, 295, 365, 354.^{1}H NMR (DMSO- d_6) d (ppm): 7.32 (1H, H-6′); 6.86 (2H, H-8, 5′), 6.63 (2H, H-3, 2′), 6.8 (1H, s, H-3), 6.28 (1H, H-6), 1.14 (3 H, CH_3 of rhamnose and an anomeric proton of glucose and rhamnose at 4.99 and 4.7 (2H, H-1″, H-1‴), broad peak at 3.6-3.1 attributed to protons of sugar moieties

^{13}C NMR (DMSO) (ppm): 163.3 (C-2), 102.9 (C-3), 182.6 (C-4), 161.5 (C-5), 99.78 (C-6), 165.5 (C-7), 94.9 (C-8), 157.44 (C-9), 102.9 (C-10), 122.1 (C-1′), 115.6 (C-2′), 145.6 (C-3′), 145.6 (C-4′), 119.3 (C-5′), 122.1 (C-6′), 105.7 (C-1′′), 73.4 (C-2′′), 76.5 (C-3′′), 71.1 (C-4′′), 79.1 (C-5′′), 68.5 (C-6′′), 113 (C-1′′′), 73.4 (C-2′′′), 72.7 (C-3′′′), 73.7 (C-4′′′), 74.3 (C-5′′′), 16.6 (C-6′′′).

***Grosheimin* (4)**

^{1}H NMR($CHCl_3$- d_6) d (ppm): 3.11 (H-1); 2.34 (H-2), 2.35 (H-4), 2.54 (H-5), 3.79 (H-6), 2.80 (H-7) 4.07 (H-8), 2.53, 1.96 (H-9), 6.27, 6.28 (H-13), o5.03, 4.75 (H-14), 1.19 (H-15) ^{13}C NMR ($CHCl_3$) (ppm): 40 (C-1), 42.8 (C-2), 220.5 (C-3), 46.8 (C-4), 50.8 (C-5), 83.3 (C-6), 48.9 (C-7), 72.7 (C-8), 42.8 (C-9), 144.6 (C-10), 137.8 (C-11), 170.5 (C-12), 123.8 (C-13), 113.9 (C-14), 13.8 (C-15).

***Solstitalin A* (5)**

^{1}H NMR($CHCl_3$- d_6) d (ppm): 2.87 (H-1); 2.32 (H-2a), 2.00 (H-2b), 2.5 (H-3), 2.9 (H-5), 4.9 (H-6), 2.8 (H-7) 2.24 (H-8a), 2.22.(H-8b), 2.56 (H-9a), 2.01 (H-9b), 4.25 (H-13), 4.84 (H-14a), 5.22 (H-14b), 5.46 (H-15a),

^{13}C NMR ($CHCl_3$) (ppm): 42.0 (C-1), 38.0 (C-2), 72.0 (C-3), 152.5 (C-4), 52.0 (C-5), 82.0 (C-6), 50.0 (C-7), 26.0 (C-8), 36.0 (C-9), 149.5 (C-10), 77.5 (C-11), 197.7 (C-12), 63.0 (C-13), 112.0 (C-14), 108.38 (C-15).

Biological activity

Radical Scavenging activity′s method ((DPPH$^{\circ}$).

Evaluation of the antioxidant activity was carried out by measuring the decolourizing capacity of each extract against stable DPPH° radical; the colour change being measured by spectrophotometer at 516 nm**. [12]**. Methanolic solutions (10^{4} - M) of DPPH° (freshly prepared), compounds to be tested, and reference compound (Trolox) were mixed, shaken vigorously, and kept 30 mins in the dark at room temperature, then the absorbance at 516 nm of the resulting solution was measured spectrophotometrically against the absorbance of a blank sample containing the same amount of methanol free from the extracts and DPPH$^{\circ}$ solution was prepared and measured. All determinations were carried out in triplicate. Any decrease in the intensity of the purple color of DPPH° solution, indicates an antioxidant activity of the extract.

The radical scavenging activity of the tested sample expressed as percent inhibition, was calculated with the following formula:

Scavenging effect % = [Control absorbance - sample absorbance /control absorbance] x 100

Rat hepatocytes monolayer culture

Isolation and preparation of rat hepatocytes monolayer culture

A primary culture of rat hepatocytes was prepared according to the Seglen method[13], which was modified by Kiso *et al*. [14], using a Wistar male rat (250–300 g). The rat was obtained from the animal house of the NRC (National Research Center, Cairo). Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals[15].

IC_{50} determination on rat hepatocytes monolayer culture

After 22–24 h, the rat hepatocyte monolayer was washed twice with phosphate buffer saline (PBS). In order to determine IC_{50}, different concentrations were prepared for each sample (250 - 1000 µg mL^{-1}). After 2 h of cells incubation with the extract, cell viability was determined using the MTT assay. The assay was performed according to the method of Mosmann, (1993) [16] modified by Carmichael *et al*. (1987) [17]. Absorbance of formasan crystals produced by viable cells was read at 540 and 630 nm dual wavelength using the Automatic Kinetic Microplate Reader (Labsystems Multiskan RC reader). Each experiment was repeated three times, and the mean absorption of each concentration was calculated. A graph plotted with *x*-axis showing the different concentrations of the extract used and the *y*-axis showing the absorbance percentage of viable cells. The IC_{50} was graphically determined from the concentration that yielded an absorption coinciding with the 50% of cells that received no extract.

Evaluation of hepatoprotective activity

Different concentrations were prepared from *Cynara cornigera* (12.5–100 µg/mL) using the serial dilutions technique by dissolving in DMSO (1% maximum concentration). For each concentration, three replicates were carried out; in addition to positive control, that was 50 µg/mL Silymarin. The plate was incubated for 2 h at 37°C and 5% CO_2, and then washed twice with PBS. A 200 µL of 25 mM paracetamol was added to each well. After 1 h of cells incubation with the paracetamol, cell viability was determined using the MTT assay. The concentration of the extract that was able to protect the cells from the hepatotoxic effect of paracetamol by 100% was considered hepatoprotective.

RESULTS AND DISCUSSIONS

Isolated compounds

By correlating with spectral data (UV, ^{1}H, ^{13}C NMR and MS) of literature values, compounds **1–5** were identified as luteolin-7-O-glucoside (cynaroside) **1**, apigenin-7-O-glucoside **2**, luteolin-7-O-rhamno glucoside (scolymoside) **3**, and two sesquiterpens i. e. grosheimin **4** and solstitalin A **5**, to our knowledge compounds 4& **5** was isolated for the first time from this species [18-21].

The different extracts obtained from *C. Cornigera* were evaluated using radical scavenging method and showed different ranges of antioxidant activities, where 80% methanol, ethyl acetate fractions, compound 1 (luteolin 7-O-glucoside) and compound 3 (luteolin -7-O-rutinoside) showed the highest antioxidant activities (88.46 %, 89.77%, 90.2% and 90.5% respectively). On the other hand apigenin 7-O-glucoside compound **2** and the two sesquiterpenes showed negative results (Table 1 and Fig. 1).

Hepatotoxicity

The viability assay was applied with a broad range of concentrations of the studied extracts of *Cynara cornigera* (from 250-1000 µg/mL) on monolayer of rat hepatocytes. It revealed that EtOAc fraction and total 80% MeOH, Luteolin-7-O- glucoside (Compound **1)** and Luteolin-7-O- rutinoside (Compound **3)** did not

show IC_{50} effect on the rat monolayer hepatocyte till a concentration of 1000 µg/mL. On the other hand; the mother liquor of *C. cornigera* showed IC_{50} at a concentration of 125 µg/mL. [Figure 2].

Evaluation of hepatoprotective activity

Some tested extracts exhibited hepatoprotective effect at a concentration of 12.5 µg/mL which were the EtOAc fraction and the methanolic extract of *C.cornigera*. At the same time, Luteolin-7-O-glucoside **1**, Luteolin-7-O-rutinoside **3** showed hepatoprotective activity at a concentration of 50, 25 µg/mL, respectively.

The mother liquor of *C.cornigera* did not protect against the induced toxicity of paracetamol exerted on the rat hepatocytes monolayer till concentration of 100 µg/mL [Figure 3].

From the hepatotxicity and hepatoprotection results we can conclude that most of the tested extracts, fractions and compounds showed a safety margin, as the hepatotoxicity dose is 20 folds or more that of the hepatoprotective dose. These results were found to be in agreement with that obtained for *Cynara scolymus* as reported [2,8,9] . The mother liquor did not show any safety margin since it has no protective effect on the hepatocyte monolayer against the toxicity of paracetamol.

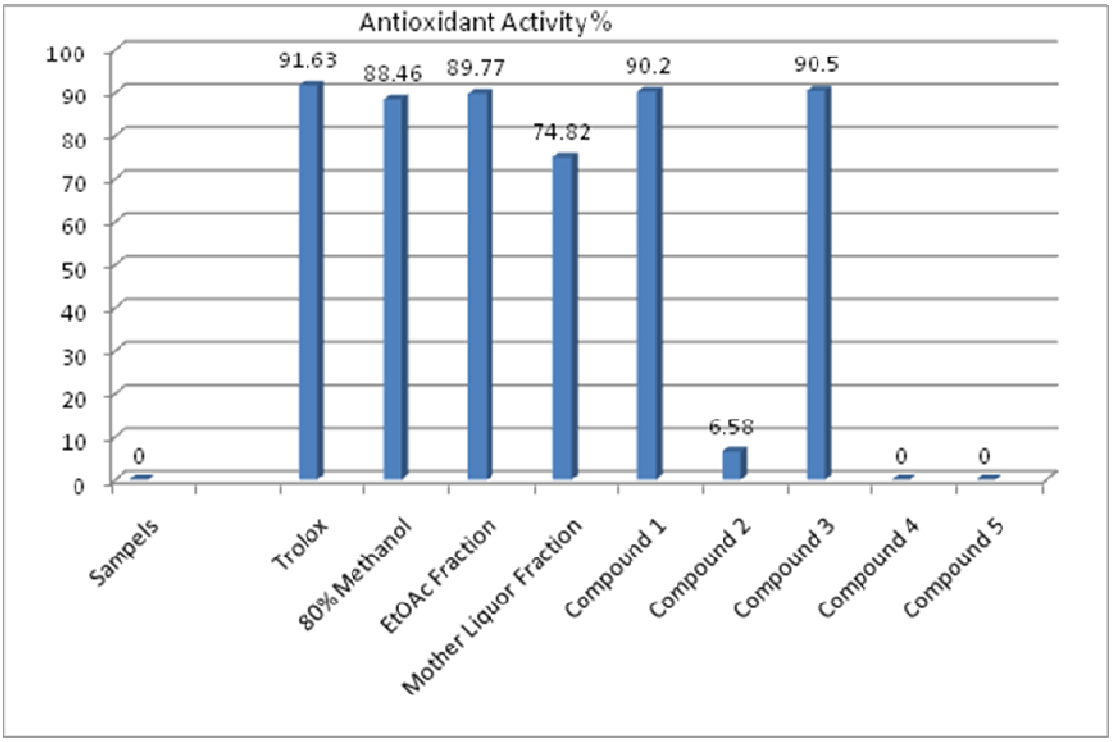


Fig. 1: Radical Scavenging Activity (expressed as absorbance percentage) of *Cynara cornigera* (Wild)

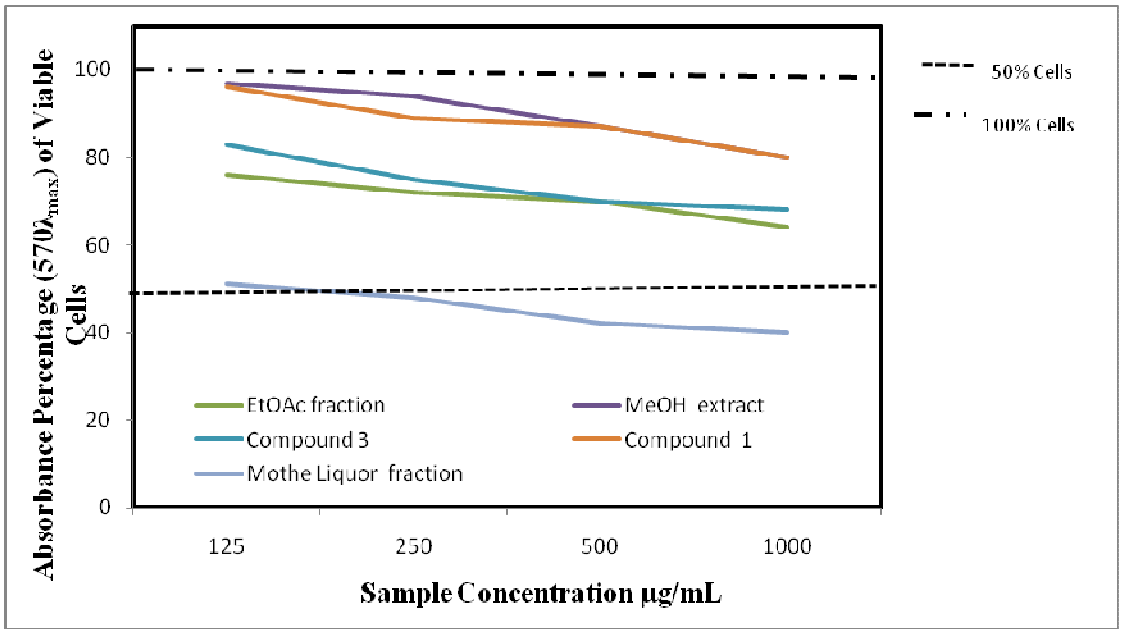


Fig. 2: Viability of monolayer of rat hepatocytes after 2 h treatment with different concentrations of the extracts using the MTT colorimetric assay

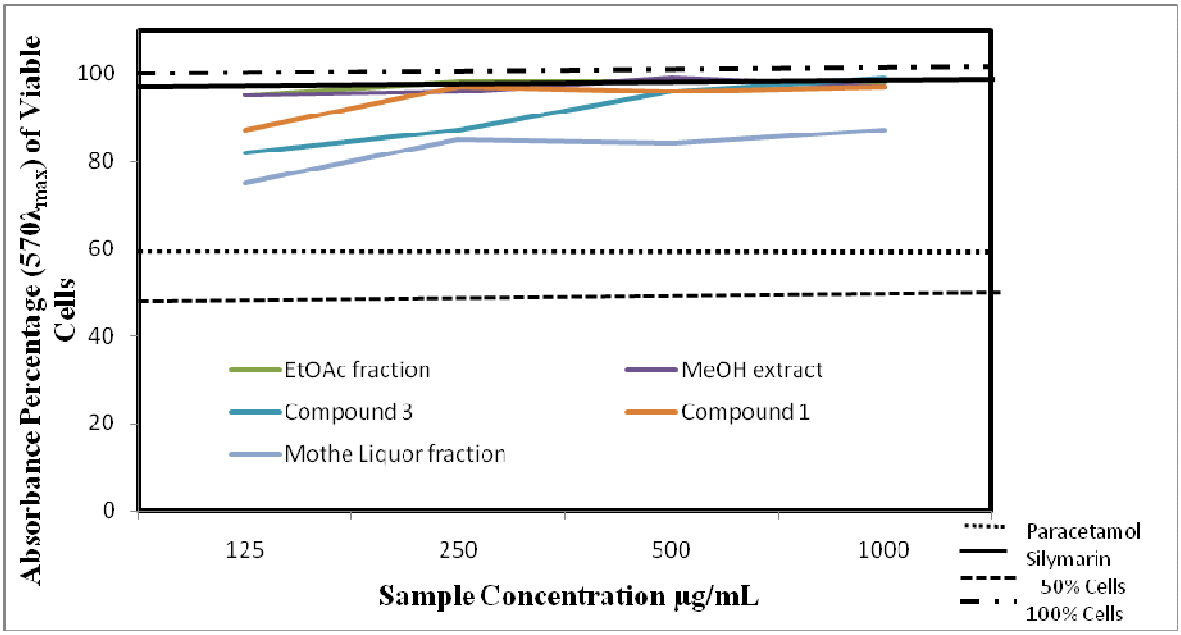


Fig. 3: Viability of monolayer of rat hepatocytes after 2 h treatment with different concentrations of the extracts followed by treatment with 25 mM paracetamol for 1 h in comparison with 50 µg silymarin as control using the MTT colorimetric assay.

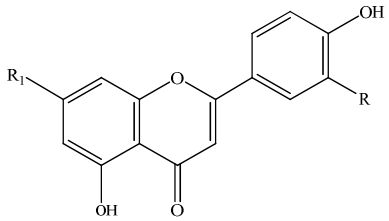
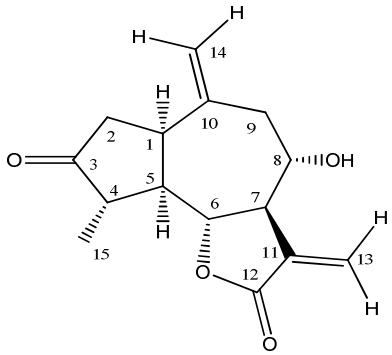


Fig. 4: Chemical structure of the isolted compounds

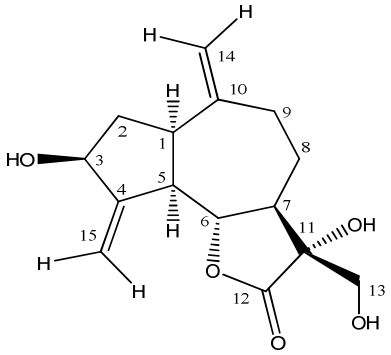
R=OH, R_1=Glucose Luteolin-7-O-glucoside (cynaroside) (1)

R=H, R_1=Glucose Apigenin-7-O-glucoside (2)

R=OH, R_1=Rhamnoglucoside Luteolin-7-O-rhamnoglucoside (scolymoside) (3)



Grosheimin (4)



Solstitalin A(5)

ACKNOWLEDGMENT

This work is partially supported by Science and Technology Development Fund STDF, Egyptian Academy of Scientific Research and Technology "ID# 245", The Authors are Grateful to the Sponsorship of the Research Centre, College of Pharmacy and the Deanship of the Scientific Research, King Saud University, Riyadh, Saudi Arabia

REFERENCES

1. Zhu XF, and Zhang HX, Flavonoids of *Cynara scolymus*. *Chemistry of Natural Compounds* 2004; 40: 600-601
2. Gebhardt R. Antioxidative and protective properties of extract from leaves of the Artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. Toxicology and Applied Pharmacology 1997; 144: 279–286.
3. Chinou I, Harvala C, Polyphenolic constituents from the leaves of two Cynara species growing in Greece. Planta Medica 1997; 63: 469–470.
4. Adzet T, Camarasa J, Carlos Laguna J, Hepatoprotective activity of polyphenolic compounds from *Cynara scolymus* against CCl_4

toxicity in isolated rat hepatocytes. Journal of Natural Products 1987; 50: 612–617.

5. Englisch W, Beckers C, Unkauf M, Zinserling V, Efficacy of Artichoke dry extract in patients with hyperlipoproteinemia. Arzneim. Forsch. (Drug Res.) 2000; 50: 260-265.
6. Hoskeri HJ, Krishna V, Ramesh BK, Badarinath DK. Prophylactic Effects of Flafervia Trinervia Extract Against Ethanol Induced Hepatotoxicity Rats. Int J Pharm Pharm Sci 2012; 4: 127-133
7. Lopez-Molina D, Heering HA, Smulevich G, Tudela J, Thorneley RNF, Garcia-Canovas F, Rodriguez-Lopez JN, Purification and characterization of a new cationic peroxidase from fresh flowers of Cynara scolymus L. J Inorg Biochem 2003; 94: 243–254
8. Fraga CG, Martino VS, Ferraro GE, Coussio JD, Boveris A, Flavonoids and Antioxidant evaluated by in vitro and in situ liver chemiluminescence. Biochemical Pharmacology 1987; 36: 717–720
9. Dasgupta B, Kalita JC, Chowdhury A, Kotoky J, Hepatoprotective Activity of *Lsonav Canescena* (Lam.) Spring on Acute Hepatotoxicity Induced by Paracetamol. Int J Pharm Pharm Sci 2012; 4: 107-112
10. Brent JA, and Rumack BH, Role of free radicals in toxic hepatic injury. J. Toxicol. Clin. Toxicol. 1993; 31: 173-196
11. Knight JA, Diseases related to oxygen-derived free radicals. Annals of Clinical and Laboratory Science 1995; 25: 111-121.
12. Nenadis N, Tsimidou M, Observations on the estimation of scavenging activity of phenolic compounds using radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH•) test. Journal of American Oil Chemical Society 2002; 79: 1191-1195.
13. Seglen PO, Preparation of isolated rat liver cells. *Methods Cell Biol.,* 1976; 13: 29-83.
14. Kiso Y, Tohkin M, Hikino H, Assay method for antihepatotoxic activity using galactosamine-induced cytotoxicity in primary-cultured hepatocytes. *J. Nat. Prod.,* 1983; 46: 841-847.
15. NIH Guide for the Care and Use of Laboratory Animals. NIH *Publication* Number. 85-23, Revised 1985, U.S. Department of Health, Education and Welfare. Research Triangle Park, North Carolina.
16. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assay. *J. Imm. Meth*, 1983; **65**: 263-271.
17. Carmichael J, DeGraff W, Gazdar A, Minna J, Mitchell J, Evaluation of tetrazolium-based semi-automated colorimetric assay: assessment of chemosensetivity testing. *Cancer Res.,* 1987; 47: 936-942
18. Wang M, James I, He K, Zheng Q-Yh, and Tadmor T, Analysis of Antioxidative Phenolic Compounds in Artichoke (Cynara Scolymus L). *Agricultur Food Chem.* 2003; 51: 601–608
19. Zhu X, Zhang H, Lo A, Phenolic Compounds from the Leaf Extract of Artichoke (*Cynara scolymus* L.) and Their Antimicrobial Activities. Journal of Agricultural and Food Chemistry 2004; 52: 7272–7278.
20. Barbetw P, Fardella G, Chiappini I, Scarcia V. and Brent JA, Rumack BH, Role of free radicals in toxic hepatic injury II. Clinical Toxicology 1993; 31: 173–196.
21. Mericli AH, and Seyhan GV, Constituents of *Cynara syriaca* Leaves. Pharmaceutical Biology, 2006; 44: 643-645.