Bull. Pharm. Sci., Assiut University, Vol. 37, Part 2, 2014, pp. 65-76.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://www.aun.edu.eg/faculty_pharmacy/index.php
e-mail: bullpharm@aun.edu.eg

PHYTOCHEMICAL INVESTIGATION OF THE AERIAL PARTS OF *CENTAURIUM SPICATUM* WITH HEPATOPROTECTIVE AND mRNA ENZYMATIC INHIBITION ACTIVITIES

Ahmed E. Allam[1*], Mohamed A. El-Shanawany[2], Enaam Y. Backheet[2] and Alaa M. Nafady[1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut 71524, Egypt*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt*

*Five compounds (**1-5**) were firstly reported from the genus Centaurium family Gentianaceae. They were identified as Lisianthoside **1**, Secoxyloganin **2**, Secologanin dimethyl acetal **3**, 1,8-Dihydroxy-3,5,6,7-tetramethoxyxanthone (Demethyleustomin) **4** and 1-Hydroxy-3,5,6,7,8-pentamethoxyxanthone (Eustomin) **5**. Structure elucidation was carried out with support of chemical and spectral analysis including 1D and 2D NMR experiments. All the isolated compounds showed promising antioxidant activity, liver microsomal enzymes reducing activity and CYP3A4 mRNA inhibition activity in the HepG2 cell line.*

INTRODUCTION

Centaurium spicatum (L.) Fritsch (*Gentianaceae*) is an annual herb occurring in Southern Europe and Northern Africa where it is used together with other *Centaurium* species such as *C. pulchellum* in traditional medicine for treatment of abdominal pain, hypertension, gallstones, kidney and ureter stones, renal colic, wounds and diabetes[1&2]. A survey of the current literatures revealed the isolation and identification of secoiridoids (sweroside, swertiamarin and gentiopicrin) and polyoxygenated xanthones from the plant[3&4]. Alkaloids of pyridine type (e.g. gentianine), spicatine and the series of amides derived from the secoiridoid glucoside swertiamarin and kantaurin were also shown to be present[3]. Among the most important pharmacological activities of the genus *Centaury* were the hepatoprotective and the antioxidant activities attributed to secoiridoides and flavonoid glycosides[5]. The hepatoprotective and CYP 450 enzymatic activities of the isolated compounds were evaluated in this paper.

EXPERIMENTAL

General experimental procedures

^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were measured on a JEOL GSX400 spectrometer in CD_3OD and DMSO-d_6. Reversed-phase high-performance chromatography experiments were undertaken on ODS columns (particle size: 5 µm, TOSO, 18×250 mm) RP-23 (5 µm; Waters). Diaion HP-20 (Mitsubishi) (Tokyo, Japan), silica gel (63-210 µm; Kanto Kagaku) and ODS (63-212 µm; Wako Pure Chemical) (Tokyo, Japan) were used for open column chromatography. Thin-layer chromatography (TLC) was carried out on silica gel (SiO_2, 60-100 mesh; Wako Pure Chemical) 60 F_{254} and RP-18 F_{254S} (Merck). Structural assignments were based on spectra resulting from one or more of the NMR experiments; ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HMQC and HMBC. The Fast Atom Bombardment (FABMS) spectra were carried out on a JEOL JMS SX-102 Mass spectrometer. Optical rotations were measured on (Horiba SEPA-3000 high-sensitivity polarimeter.

Received in 24/6/2014 & Accepted in 3/12/2014

*Corresponding author: Ahmed E. Allam, E-mail: aallam81@yahoo.co.uk

Plant material

C. spicatum (L.) Fritsch (*Gentianaceae*) aerial parts were collected in May 2009 from New Valley, 200 km Southwest of Assiut City, Egypt. The plant was identified and authenticated by Prof. Dr. A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University.

Extraction and isolation

Air-dried *C. spicatum* aerial parts (2 kg) were extracted thrice with MeOH (5 L each) at room temperature. The extracts were combined and filtered through filter paper (Advantec MFS Incorporated). The extracts were removed under reduced pressure using rotary evaporator at 40°C to yield the methanol extract (550 mg) which was mixed with distilled water and partitioned between chloroform, ethyl acetate and *n*-butanol (1 L each) to give the chloroform fraction (163 g), ethyl acetate fraction (80 g), *n*-butanol fraction (100 g) and the rest aqueous fraction (200 g). The ethyl acetate fraction was in turn partitioned between (methanol-water 90%) and *n*-hexane to give 90% methanol fraction (50 g) and *n*-hexane fraction (20 g). All fractions were screened for the antioxidant, hepatoprotective and *m*RNA enzymatic inhibition activities where noticed that the *n*-butanol fraction (100 g) is the most active fraction and hence, it was sub- fractionated on Diaion HP-20 column using water (2 L) and methanol (25%, 50%, 75% and 100%) (2 L each). The fraction eluted by 50% methanol from the *n*-butanol fraction) (33.4 g) was further partitioned by chromatography on ODS column (80×200 mm) (Cosmosil 140 C_{18} PREP, Nacalai Tesque, Tokyo, Japan) using mobile phase systems of CH_3CN-H_2O (10, 25, 40, 50, 70 and 90% v/v; elution volume: 1.5 L of each) to give six corresponding fractions. The fraction eluted with 40% CH_3CN (3.8 g) was further chromatographed by column chromatography on silica gel and eluted stepwise gradient with $CHCl_3$ - MeOH (ratios of 9:1, 6:1, 4:1, 3:1 and 1:1, v/v elution volume: 200 ml each) to give five corresponding fractions. The fraction eluted with 6:1 $CHCl_3$ - MeOH was further partitioned by preparative HPLC, ODS column: C30 UG-5 ODS (20 mm× 250 mm) particle size: 5 µm, flow rate: 6 ml/min. (Develosil, Nacalai Tesque, Tokyo, Japan) equipped with a UV detector (210 nm). The mobile phase was 20% CH_3CN in H_2O. This resulted in the isolation of compounds **1-4**. These preparative HPLC conditions were also used after gradually increasing the mobile phase to 50% CH_3CN in H_2O to separate the same fraction giving compound **5.**

Cytotoxic assay (MTT assay)

This method is a colorimetric assay for assessing cell viability[6]. To determine the cytotoxic activity of the tested samples, THP-1 cells (180 µl) were seeded in 96-well plates at 1.0×10^5 cells per well with tested samples (purity > 93%) (20 µl in DMSO/ PBS) at various concentrations. After 48-h cultivation, the supernatants were removed, non-adherent cells (THP-1) incubated with the tetrazolium dye; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), 10 µl, 5 mg/mL in PBS for 4 h and then solubilized with 10% (w/v) sodium dodecyl sulfate (SDS; in 60% [v/v] dimethyl formamide) solution (100 µl) for 18 h. The absorbance was measured at 570 nm using a microplate reader and the cytotoxicity calculated by comparing absorbance with that of the non-treated control culture. Cell growth curve was graphed using statistical analysis software (Kaleida Graph version 4.00; Synergy Software) and IC_{50} values calculated using simple linear regression.

DPPH radical scavenging activity

DPPH assay was performed by a method previously reported by Kumar *et al*.[7]. The tested samples (100 µl) at different concentrations in MeOH and 1.0×10^{-4} M DPPH (Wako) (Tokyo, Japan) in MeOH (300 µl) were added to 96-well microtiter plate. The plate was shaken for 1 min on a plate shaker and incubated for 30 min at room temperature in the dark. After incubation, the absorbance was recorded at 517 nm[8-10]. The tested samples at different concentrations without DPPH solution were used as a blank control to eliminate the influence of sample color. Ascorbic acid was used as a positive control[7] and DPPH solution in MeOH served as a negative control.

Data analysis

Ratio (percentage of control) of DPPH was determined as mean ± SD. Statistical significance was determined by Dunnett's

multiple test after one-way analysis of variance (ANOVA) with comparison to a control group using statistical analysis software (Kaleida Graph ver. 4.00). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Results

Compound 1: Obtained as white amorphous powder (12 mg), $[\alpha]_D^{31.8}$ -412° (c= 0.333, MeOH). ^{1}H-, ^{13}C-NMR (400, 100 MHz, CD_3OD): (Tables 1 and 2). FAB MS at *m/z*: 717 $[M+H]^+$ $C_{32}H_{44}O_{18}$.

Compound 2: Obtained as white crystals (17 mg), m.p. 142-144°C. ^{1}H-, ^{13}C-NMR (400, 100 MHz, CD_3OD): (Tables 1 and 2). FAB MS at *m/z*: 405 $[M+H]^+$ $C_{17}H_{24}O_{11}$.

Compound 3: Obtained as white amorphous powder (12 mg), $[\alpha]_D^{28.0}$ -102.7° (c= 0.45, MeOH). ^{1}H-, ^{13}C-NMR (400, 100 MHz, CD_3OD): (Tables 1 and 2). FAB MS at *m/z*: 433 $[M-H]^-$ $C_{19}H_{30}O_{11}$.

Compound 4: Obtained as pale yellow needles (7 mg), m.p. 220-221°C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 11.95 (1H, s, 1-OH) and 11.87(1H, s, 8-OH), 6.48 (1H,d, *J*= 2.4 Hz, H-4), 6.33 (1H, d, *J*= 2.4 Hz, H-2), 4.12 (3H, s, 7-OCH_3), 3.92 (3H, s, 6-OCH_3), 3.91(3H, s, 3-OCH_3) and 3.88 (3H, s, 5-OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): Table 2. FAB MS at *m/z*: 349 $[M+H]^+$ $C_{17}H_{16}O_8$.

Compound 5: Obtained as pale yellow needles (6 mg), m.p. 83-84.5°C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 11.78 (1H, s, 1-OH), δ_H 6.69 (1H,d, *J*= 2.4 Hz, H-4), 6.40 (1H, d, *J*= 2.4 Hz, H-2), 4.06 (3H, s, 8-OCH_3), 3.89 (3H, s, 6-OCH_3), 3.86 (3H, s, 3-OCH_3), 3.81(3H, s, 7-OCH_3) and 3.80 (3H, s, 5-OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): Table 2. FAB MS at *m/z*: 363 $[M+H]^+$ $C_{18}H_{18}O_8$.

Table 1: ^{1}H -NMR assignments for compounds **1-3** (CD_3OD, 400 MHz).

Proton No.	Cpd. 1 (part a)	Cpd. 1 (part b)	Cpd. 2	Cpd. 3
1	5.32, 2H, br.s	5.32, 2H, br.s	5.43, d, 4.1	5.52, t, 5.0
2	–	–	–	–
3	7.50, br.s	7.20, br.s	7.59, brs	7.44, d, 3.2
4	–	–	–	–
5	3.09, m	3.26, m	3.81, m	2.93, ddd, 7.3, 6.8, 5.5
6	3.08, m 2.17, m	1.61, m 1.98, m	*α*: 2.20, dd, 16.8, 9.6 *β*: 3.03, dd, 16.8, 4.4	1.64, ddd, 14.1, 7.8, 4.1
7	2.8, 2H, t, 12.0 4.52, d, 12.0	4.28, 2H, t, 12.0 4.36, d, 12.0	–	4.50, dd, 7.3, 4.1
8	5.47, dd, 9.2, 18.0	5.55, dd, 9.9, 18.0	5.62, ddd, 9.2 (*cis*, H-8, H-10_a) 16.8 (*trans*, H-8, H-10_b) 9.6 (H-8, H-9)	5.77, m
9	2.58, ddd, 5.1, 9.2, 15.0	2.86, m	2.92, d, 9.6	2.68, ddd, 8.7, 5.5, 5.0
10	5.14, 2H, m	5.22, 2H, m	5.38, d, 9.2 (*cis*, H-10_a, H-8) 16.8 (*trans*, H-10_b, H-8)	5.29, m
11- $COOCH_3$	–	–	3.31, 3H, s	3.71, 3H, s
12a	–	–	–	3.31, 3H, s
12b	–	–	–	3.31, 3H, s
1'	4.61, d, 7.2	4.51, d, 7.2	4.60, d, 7.9	4.68, d, 7.7
2'	3.25, m	3.41, m	3.13-3.18, m	3.36, m
3'	3.29, m	3.25, m	3.13-3.18, m	3.21, m
4'	3.41, m	3.26, m	3.13-3.18, m	3.24, m
5'	3.41, m	3.30, m	3.13-3.18, m	3.92, m
6'	6_a: 4.05, dd, 4.5, 11.6 6_b: 4.52, d, 11.6	6_a: 3.65, dd, 4.5, 11.6 6_b: 3.75, d, 11.6	a: 3.85, dd, 2.4, 12.0 b: 3.35, m	3.92, m 3.66, m

Table 2: ^{13}C -NMR assignments for compounds **1-5** (Cpds. **1, 2, 3** in CD_3OD and compounds **4, 5** in DMSO-d_6, 100 MHz).

Carbon No.	Chemical shift ^{13}C - NMR (δ, mult.)					
	Cpd. 1 (a)	**Cpd. 1 (b)**	**Cpd. 2**	**Cpd. 3**	**Cpd. 4**	**Cpd. 5**
1	98.0, d	97.0, d	97.4, d	97.8, d	162.7, s	161.7, s
2	–	–	–	–	97.7, d	97.8, d
3	153.8, d	152.0, d	153.6, d	153.1, d	166.9, s	166.9, s
4	105.0, s	111.0, s	110.1, s	111.6, s	93.0, d 4a = 157.6, s 4b = 140.7, s	93.1, d 4a = 154.0, s 4b = 145.3, s
5	28.3, d	28.7, d	28.3, d	29.3, d	124.6, s	124.6, s
6	35.4, t	25.9, t	34.9, t	33.1, t	132.5, s	149.3, s
7	69.7, t	69.7, t	176.3, s	104.3, d	150.4, s	147.5, s
8	133.2, d	134.1, d	134.5, d	135.8, d	154.3, s 8a = 109.0, s 8b = 102.2, s	142.2, s 8a = 112.8, s 8b = 103.0, s
9	43.9, d	44.6, d	45.2, d	45.2, d	183.9, s	183.3, s
10	120.7, t	121.0, t	121.3, t	119.8, t	3-OCH_3 = 55.9, q	3-OCH_3 = 56.3, q
11	168.5, s	173.8, s	170.2, s	169.0, s	5-OCH_3 = 61.2, q	5-OCH_3 = 61.0, q
$COOCH_3$	–	–	51.9, q	51.7, q	6-OCH_3 = 62.1, q	6-OCH_3 = 61.5, q
12a	–	–	–	53.9, q	7-OCH_3 = 61.7, q	7-OCH_3 = 61.0, q
12b	–	–	–	52.5, q	–	8-OCH_3 = 61.7, q
1'	99.8, d	99.8, d	99.9, d	100.0, d		
2'	74.4, d	74.2, d	74.6, d	74.5, d		
3'	77.5, d	75.6, d	77.9, d	77.9, d		
4'	71.0, d	71.0, d	71.5, d	71.4, d		
5'	78.2, d	78.0, d	78.3, d	78.3, d		
6'	62.6, t	62.3, t	62.7, t	62.7, t		

Discussion

The biological guided fractionation of the *n*-butanol fraction of the methanolic extract of *C. spicatum* (L.) Fritsch aerial parts have been resulted in the isolation of five compounds including three secoiridoids (**1-3**) and two xanthones (**4** and **5**) (Fig. 1).

Investigation of ^{1}H-, ^{13}C- and DEPT ^{13}C-NMR spectra of compound **1** (Tables 1 and 2) concluded the presence of a sweroside dimer which was obvious from the presence of two acetalic protons at δ_H 5.32 (2H, brs, H1-part a) and 5.32 (2H, brs, H1-part b) in addition to two vinylic protons at δ_H 5.14 (2H, m, H10-part a) and 5.22 (2H, m, H-10, part b). This was confirmed from ^{13}C-NMR spectrum by the appearance of signals at δ_c (98.0, d, C-1, part a) and δ_c (97.0, d, C-1, part b). Regarding sugar moiety, it had been found that ^{1}H-NMR spectrum displayed signals at δ_H 4.61 (1H, d, *J*= 7.2 Hz, H1'- part a) and 4.51 (1H, d, *J*= 7.2 Hz, H1'- part b) assignable to a couple of anomeric protons which was further indicated from ^{13}C-NMR signals at δ_c 99.8 (d, C1'-part a) and 99.8 (d, C1'-part b). These data revealed the existence of a pair of separate glucopyranoside moieties attached to C-1 of both (a) and (b) parts[11&12]. Configuration of the sugar moiety was concluded to be of *β* type from the coupling constant of H-1' of both parts (*J*= 7.2 Hz of both parts). From the 2 DNMR correlations including both HMQC and HMBC experiments, in addition to the above mentioned data and upon comparison with literature data[11-13], compound **1** was identified as a symmetrical secoiridoid dimer Lisianthoside which was firstly reported from the genus *Centaurium*.

The ^{1}H-NMR spectrum of compound **2** (Table 1) showed the presence of an olefinic proton signal at δ_H 7.59 (1H, brs, H-3) in addition to an acetal proton at δ_H 5.43 (1H, d, *J*= 4.1 Hz, H-l) and a signal at δ_H 5.38 (2H, d, *J*= 9.2 Hz, H-10) which was indicative to a

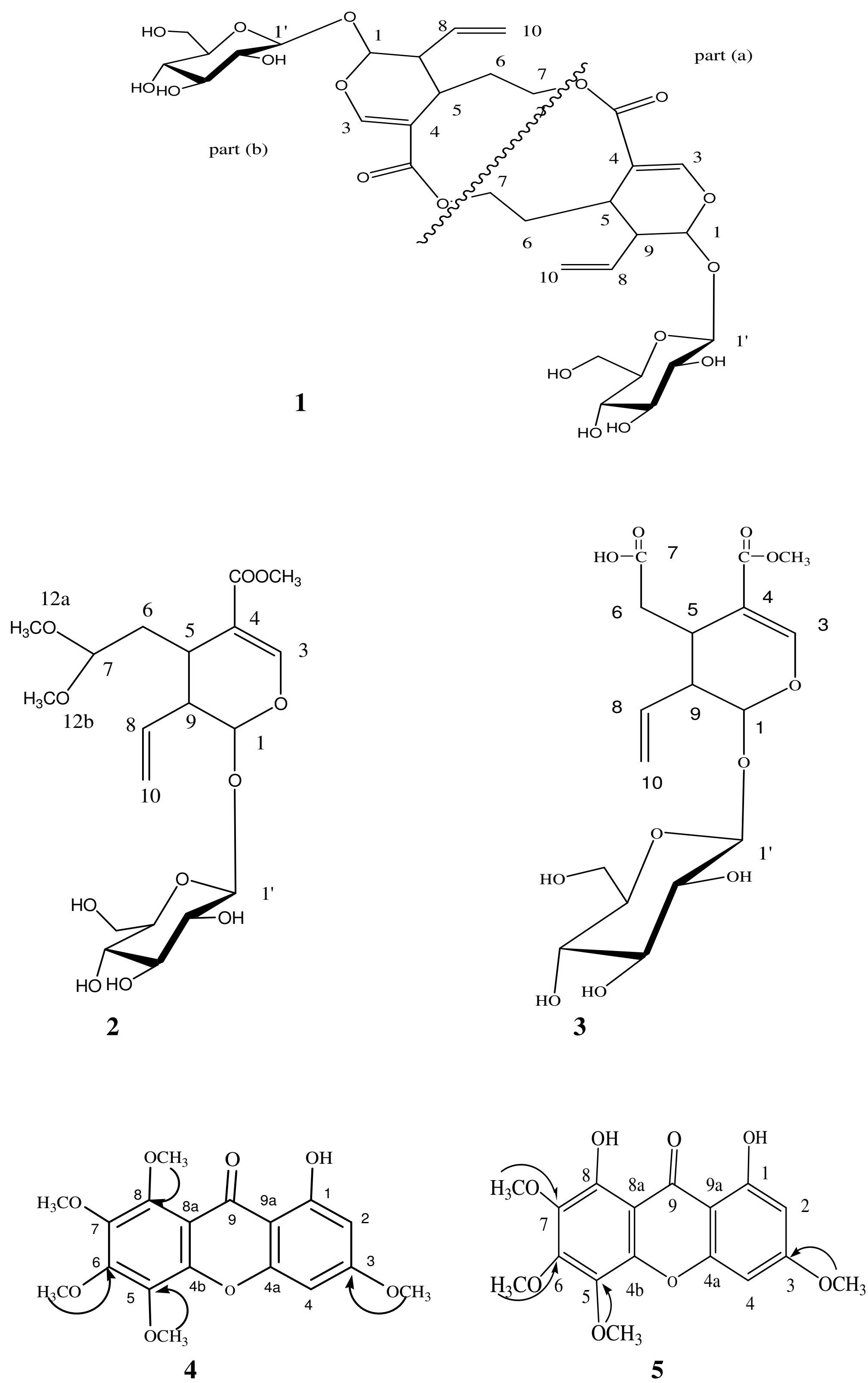


Fig. 1: Structure of compounds **1-5**.

vinylic group. A signal at δ_H 2.20 (1H, dd, J= 16.8 and 9.6 Hz) was assigned to the proton of C-6 exists at α-position while the proton presents at β-position showed a signal at δ_H 3.03 (1H, dd, J= 16.8 and 4.4 Hz) and an anomeric proton signal for β- glucopyranosyl unit appeared at δ_H 4.60 (1H, d, J= 7.9 Hz). ^{13}C-NMR spectrum (Table 2) displayed 17 signals including signals which were assigned to one glucose moiety. The structure was well confirmed by HMQC and HMBC experiments and hence, compound **2** could be identified as secoxyloganin by comparison with the literature data[14] which was the first report from the genus *Centaurium*.

The ^{1}H-NMR spectrum of compound **3** (Table 1) showed the presence of an olefinic proton signal at δ_H 7.44 (1H, d, J= 3.2 Hz, H-3). Furthermore, an acetal proton signal at δ_H 5.52 (1H, t, J= 5.0 Hz, H-1) and a signal for a vinyl group at δ_H 5.29 (2H, m, H-10). In addition, three methoxyl singlets were observed at δ_H 3.71 (3H, s, H-11), 3.31 (3H, s, H-12a) and 3.31 (3H, s, H-12b). The identification of compound **3** was well confirmed from both HMQC and HMBC experiments and it was characterized as secologanin dimethyl acetal secoiridoid[13] which was first report from the genus *Centaurium*.

The ^{1}H-NMR spectrum of compound **4** (Table 1) showed signals at δ_H 11.95 (1H, s, 1-OH) and 11.87(1H, s, 8-OH), in addition to the presence of two aromatic protons at δ_H 6.48 (1H, d, J= 2.4 Hz, H-4) and 6.33 (1H, d, J= 2.4 Hz, H-2). Also, the spectrum showed signals indicated the presence of four methoxy groups at δ_H 4.12, 3.92, 3.91 and 3.88. ^{13}C-NMR spectrum (Table 2) exhibited signals characteristic to xanthone compound[15]. The sites of the four methoxy groups were decided to be at C-3, C-5, C-6 and C-7 from the long range HMBC experiment (Fig. 1). Hence, compound **4** could be identified as 1,8-Dihydroxy-3,5,6,7-tetramethoxyxanthone (Demethyleustomin)[15] which was first report from the genus *Centaurium*.

The ^{1}H- and ^{13}C-NMR spectral data of compound **5** (Tables 1 and 2) were similar to those of compound **4** except for the replacement of a hydroxyl group at position C-8 by a methoxyl one (δ_H 4.06 (3H, s, 8-OCH_3); δ_c 61.7 (q, 8-OCH_3). The structure was well confirmed from both HMQC and HMBC experiments (Fig. 1). Hence, compound **5** could be identified as 1-Hydroxy-3,5,6,7,8-Pentamethoxyxanthone, (Eustomin)[16] which was first report from the genus *Centaurium*.

The antioxidant activity (DPPH assay)

The antioxidant activity of the isolated compounds was measured by using DPPH radical formation assay (Table 3). In the DPPH assay, the isolated compounds **1-5** exhibited free radical scavenging activity with IC_{50} values of 1.37 and 1.42 μM for compound **1** and compound **2** respectively, 3.62, 3.29 and 1.87 μM for compounds **3-5** respectively. All tested compounds showed strong activities comparing with that of the positive control ascorbic acid (12 μM).

Table 3: DPPH radical scavenging activity of compounds **1-5** from *C. spicatum* aerial part.

Compounds	IC_{50} (μM) [a]	*n*
1	1.37	4
2	1.42	4
3	3.62	4
4	3.29	4
5	1.87	4
Ascorbic acid	12.0	4

IC_{50} values were determined by regression analysis and expressed as the mean of four replicates.

Hepatoprotective effect of secoiridoids (compounds 1-3) isolated *Centaurium spicatum*

It is important to clarify that SGOT and SGPT levels do not reflect the function of the liver, even though they are referred commonly to as liver function tests. They only are used to detect inflammation due to injury or damage to the liver from any source. The most important transaminases identified are glutamate-oxaloacetate transaminase (GOT) glutamate-pyruvate transaminase (GPT). Increased levels of SGOT and SGPT are found in cases of myocardial infarction, viral hepatitis, toxic liver necrosis, cirrhosis and malignant infiltration of the liver[17&18]. In this study, kits utilize the spectrophotometric method of

Karmen[19] where 2,4 dinitro phenyl hydrazine is used to convert both oxaloacetate and pyruvate to the corresponding 2,4-dinitrophenyl-hydrazine derivatives which can be measured spectrophotomerically at 555 nm. Liver diseases remain one of the serious health problems[20]. Modern medicines have little role to alleviation of hepatic diseases and the plant-based preparations which are chiefly available medicines employed for the treatment of liver disorders[21]. The present study was aimed to evaluate the hepatoprotective and antioxidant activity in addition to CYP450 inhibition activity of *m*RNA of some secoiridoids and xanthones isolated from *Centaurium spicatum* in mice within 8 h through intravenous dose of 1.5 mg/kg body weight and silymarin in a dose of 50 mg/kg body weight as appositive control on mice liver damage induced by Concanavalin A[22] (Table 4, Fig. 2).

It is noted from (Table 4, Fig. 2) that the tested secoiridoids (compounds **1-3**) have transaminases inhibitory activity with different values (85%, 88%, 84%) for SGOT and (94%, 98%, 97%) for SGPT, respectively.

CYP3A4 inhibitory activity (enzymatic activity)

Principle of assay

Vivid® CYP450 Screening Kits enable rapid measurement of interactions between drug candidates and cytochrome P450 enzymes using a simple "mix-and-read" fluorescent assay (Fig. 3) that is designed for high-throughput screening in multiwell plates (Fig. 3). Test compounds are analyzed by their capacity to inhibit the production of a fluorescent signal in reactions using recombinant CYP450 isozymes and specific Vivid® CYP450 Substrates[16-18]. Screening Kits are designed to assess metabolism and inhibition of the predominant human P450 isozymes involved in hepatic drug metabolism: CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5. The Vivid® Substrates are metabolized by a specific CYP450 enzyme into products that are highly fluorescent in aqueous solutions[23].

It was concluded from figure 4 that, the inhibitory activity of the different fractions *C. spicatum* aerial parts being greater than 100 µg, both 90% methanol and *n*-butanol fractions have a high CYP3A4 inhibition activity (> 75% and 90% with IC_{50}= 64.2 and 3.4µg/mL respectively) while *n*-hexane has no apparent activity. The correlations between the concentrations and the inhibition activity was significant where r= 0.99 and 0.95 respectively.

Table 4: Results of the transaminases inhibition activity of compounds **1-3**.

Parameters	SGOT (IU/L)	SGPT (IU/L)
Normal	46.92 ± 5.2	43.52 ± 4.25
Concanavalin A control at 1.5 m/kg bw	428.2 ± 2.25	520.1 ± 1.1
Silymarin (50 mg/kg bw) + Concanavalin A control at 1.5 m/kg bw	57.1 ± 3.45	48.5 ± 3.25
Compound **1** (100 mg/kg bw) + Concanavalin A control at 1.5 mg/kg bw	71.5 ± 6.24	20.2 ± 4.3
Compound **2**(100 mg/kg bw) + Concanavalin A control at 1.5 mg/kg bw	60.4 ± 5.3	22.8 ± 2.6
Compound **3** (100 mg/kg bw) + Concanavalin A control at 1.5 m/kg bw	53.4 ± 1.2	14.9 ± 3.12

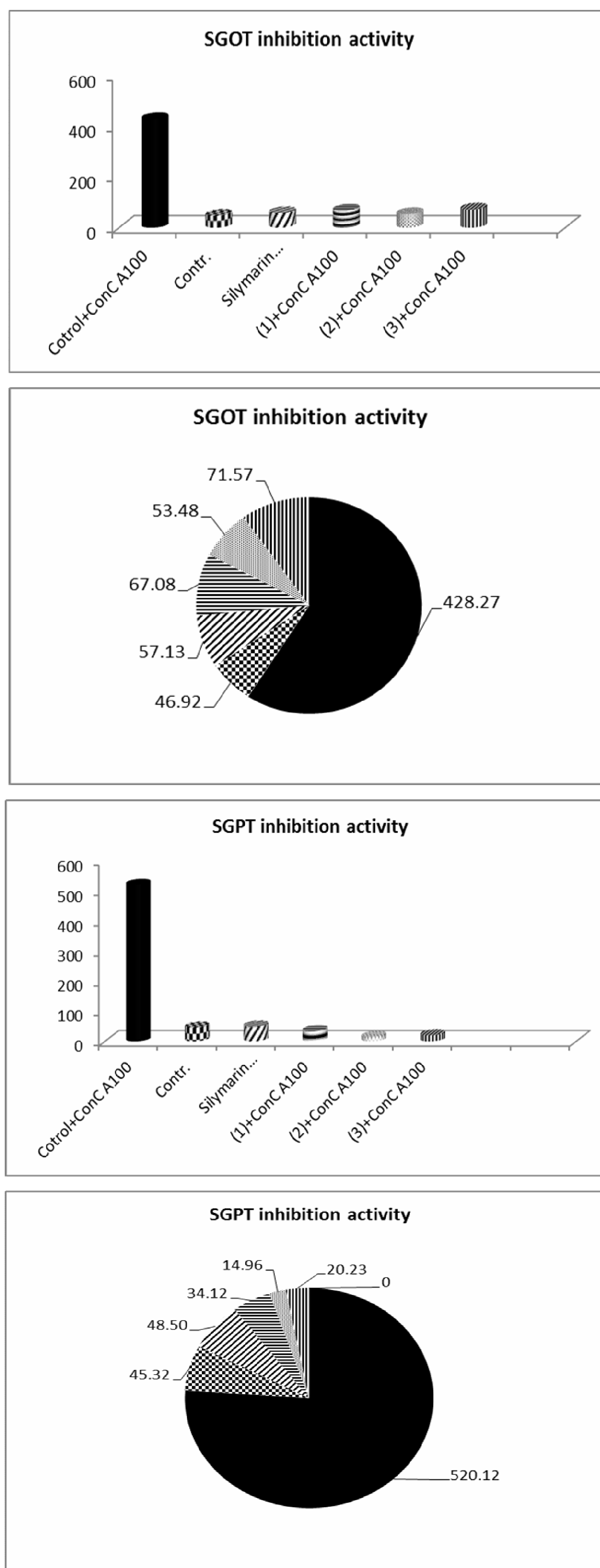


Fig. 2: Results of the transaminases inhibition activity of compounds **1-3**.

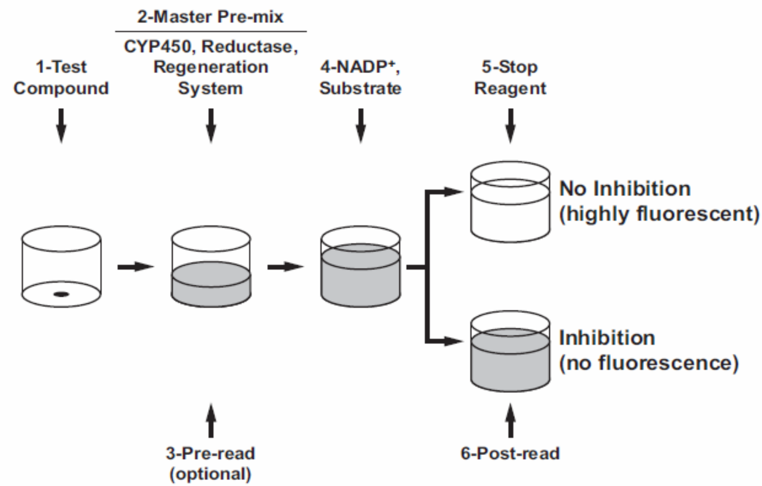
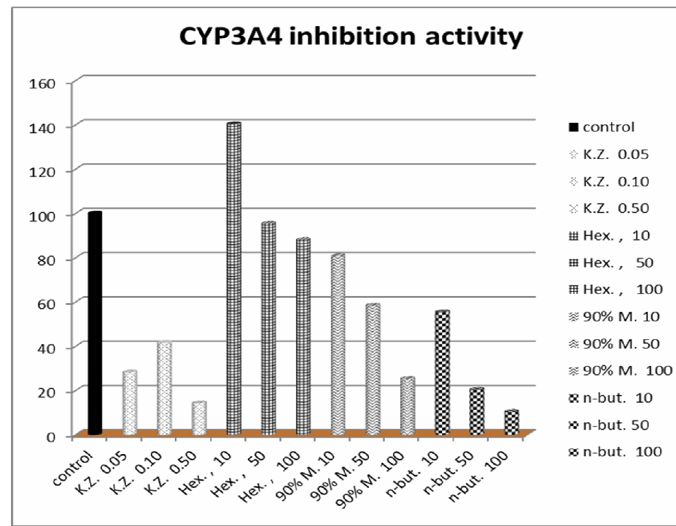
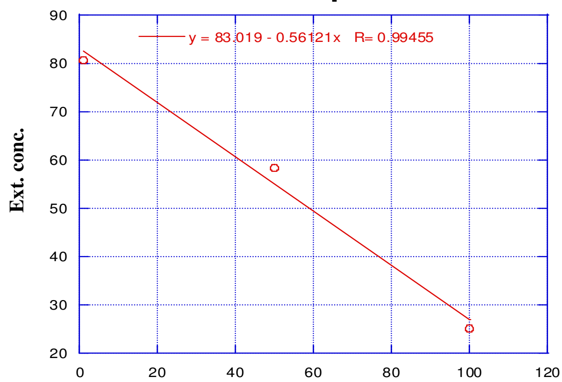


Fig. 3: Schematic representation of an endpoint Vivid® CYP3A4 assay.

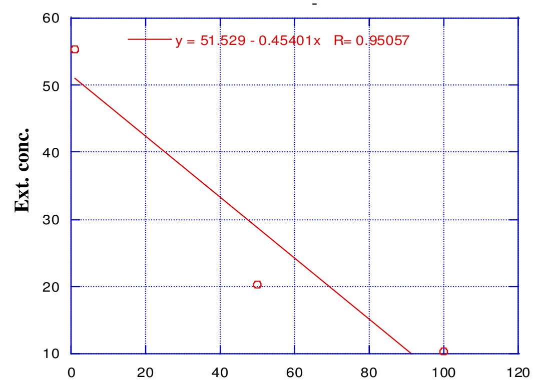


Note: K.Z. = Ketoconazole



CYP3A4 inhibitory activity

IC_{50} of 90% M.= 64.2 µg



CYP3A4 inhibitory activity

IC_{50} of *n*-but.= 3.4 µg

Fig. 4: CYP3A4 inhibition activity of *C. spicatum* of the aerial part fractions.

Acknowledgment

This work was supported in part by Pharmacognosy and Chemistry of Natural Products, School of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan, for which we are thankful.

REFERENCES

1- M. El-Shanawany, M. Makboul and A. Abdel Baky, "Phytochemical study of *Centaurium pulchellum* (SW) Druce growing in Egypt", ***Bull. Pharm. Sci. Assiut Univ.***, 12, 416-421(1989).

2- M. El-Shanawany, A. Samir, A. Gamal, M. Alaa and R. Sabrin, "A new xanthone from the roots of *Centaurium spicatum* L", ***Phytochem. Lett.***, 4 (2), 126-128 (2011).

3- W. Van der Sluis and R. Labadie, "Secoiridoids and xanthones in the genus *Centaurium*", ***Planta Med.***, 41 (3), 221-231(1981).

4- D. Bishay, S. Ross and P. Hylands, "Alkaloids and glycosides from *Centaurium spicatum*", ***ibid.***, 37 (11), 253-258 (1979).

5- A. A. Shahat, "Isolation, Complete NMR Data and Hepatoprotective Activity of Sweroside as Major Constituents of *Centaurium spicatum* (L.) Growing in Egypt", 1st International Conference of the Arab Society for Medical Research, Nov. 7-9 (2006).

6- M. V. Berridge and A. S. Tan, "Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) Subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction", ***Arch. Biochem. Biophys.***, 303, 474-482 (1993).

7- V. Kumar and M. Mohan, "Physicochemical status and primary productivity of Ana Sagar lake, Ajmer (Rajasthan), India", ***Univ. J. of Envir. Res. and Tech.***, 1, 286-292 (2011).

8- T. Berkan, L. Ustünes, F. Lermioglu and A. Ozer, "Antiinflammatory, analgesic and antipyretic effects of an aqueous extract of *Centaurium erythraea*", ***Planta Med.***, 57 (1), 34-37 (1991).

9- P. Valentão, F. Areias, J. Amaral, P. Andrade and R. Seabra, "Tetraoxygenated xanthones from *Centaurium erythraea*", ***Nat. Prod. Lett.***, 14, 319-323 (2000).

10- K. Javidnia, L. Dastgheib, S. Mohammadi and A. Nasiri, "Antihirsutism activity of Fennel (fruits of *Foeniculum vulgare*) extract". A double-blind placebo controlled study, ***Phytomed.***, 10, 455-458 (2003).

11- M. Hamburger, M. Hostettmann, H. Stoeckli, P. N. Solis, M. P. Gupta and K. Evans Hostettmann, "A novel type of dimeric secoiridoid glycoside from *Lisianthius jefensis* Robyns et Elias", ***Helv. Chim. Acta.***, 73 (7), 1845-1852 (1990).

12- T. Takao, T. Yukiko and N. Naotaka, "Two dimeric secoiridoid glucosides from *Jasminum polyanthum*", ***Phytochemistry***, 41, 1341-1345 (1996).

13- J. Hewett and R. Roth, "Hepatic and extrahepatic pathobiology of bacterial lipopolysaccharides", ***Pharmacol. Rev.***, 45, 382-411 (1993).

14- C. Adriana, V. Robert, W. Alfons and R. Lefeber, "The iridoid glucoside secologanin is derived from the novel triose phosphate/pyruvate pathway in a *Catharanthus roseus* cell culture", ***FEBS Lett.***, 434, 413-416 (1998).

15- V. Valentão, B. Paula, E. Andrade and S. Eurico, "Isolation and structural elucidation of 5-formyl-2,3-dihydroiso-coumarin from *Centaurium erythraea* aerial parts", ***J. Agric. Food Chem.***, 50 (3), 460-463 (2002).

16- T. Lamberto, M. Francesca, M. Cometa and N. Marcello, "Isolation of secoiridoid artifacts from *Lonicera japonica*", ***J. Nat.* Prod.**, 58 (11), 1756-1758 (1995).

17- W. Watson, Y. Zhao and R. Chawla, "S-adenosylmethionine attenuates the lipopolysaccharide-induced expression of the gene for tumor necrosis factor alpha", ***Biochem. J.***, 342, 21-25 (1999).

18- P. Kubes and D. McCafferty, "Nitric oxide and intestinal inflammation", ***Am. J. Med.***, 109, 150-158 (2000).

19- A. Karmen, F. blewski and J. LaDue, "Transaminase activity in human blood", ***J. Clin. Invest.***, 34, 126-131 (1955).

20- P. Baranisrinivasan, C. Elumalai, S. Sivaakumar, T. Viviyan and E. David, "Fatty acid profile tocopherol content and total antioxidant activity of oil extracted from *Nigella sativa* seeds", ***Int. J. Pharmacol.***, 5, 268-272 (2009).

21- S. Raju, S. Jayalakshmi, S. Usharani and K. Sreeramulu, "Induction of systemic acquired resistance in susceptible and resistant cultivars of chickpea (*Cicer arietinum* L) genotypes: A comparative study in response to salicylic acid and spermine", ***Physiol. Mol. Biol. Plants***, 13, 27-36 (2007).

22- G. Tiegs, J. Hentschel and A. Wendel, "A T cell-dependent experimental liver injury in mice inducible by concanavalin A", ***J. Clin. Invest.***, 90 (1), 196-203 (1992).

23- L. Cohen, H. Einolf, V. Fischer, L. Gan, S. Grimm and J. Kao, "*In-vitro* drug interaction of cytochrome P450: An evaluation of fluorogenic to conventional substrate", ***Drug Metab. Dispos.***, 31, 1005-1015 (2003).

نشرة العلوم الصيدليـــة
جامعة أسيوط

دراسة فيتوكيميائية للأجزاء العلوية لنبات السنتاوريوم ذات التأثير الواقى للكبد والمثبطة لعمل إنزيمات الحض النووى الرسول

أحمد عز الدين عبد اللاه علام[١] – محمد احمد الشنوانى[٢] – إنعام يونس بخيت[٢] – علاء محمد حسن نفادى[١]

[١]قسم العقاقير ، كلية الصيدلة ، جامعة الأزهر ، أسيوط ٧١٥٢٤ ، مصر

[٢]قسم العقاقير ، كلية الصيدلة ، جامعة أسيوط ، أسيوط ٧١٥٢٦ ، مصر

تم فصل خمسة مركبات لأول مرة من جنس نبات السنتاوريم والتابع للعائلة الجنشيانية وهى: ليثيانثوزايد (١) ، سيكوكسى لوجانين (٢) ، سيكولوجانين داى ميثيل أسيتال (٣) ، ١،٨- داى هيدروكسى-٣،٥،٦،٧- تيترا ميثوكسى زانثون (دى ميثيل أوستيومين) (٤) ، ١-هيدروكسى-٣،٥،٦،٧،٨- بنتا ميثوكسى زانثون (أوستيومين) (٥). وقد تم التعرف على هذه المركبات بالطرق الكيميائية والطيفية المختلفة. وقد أظهرت تلك المركبات نشاطا مضادا للأكسدة بالإضافة إلى خفض نشاط الإنزيمات الكبدية كما وجد أن لها تأثيرا كبيرا على تثبيط نشاط السيتوكروم ب ٣ أ فى خلايا HepG2.