Journal of Pharmacy & Pharmacognosy Research
E-ISSN: 0719-4250
editor@jppres.com
Asociación de Académicos de Ciencias Farmacéuticas de Antofagasta
Chile

Allam, Ahmed E.; Nafady, Alaa M.; El-Shanawany, Mohamed A.; Takano, Fumide; Ohta, Tomihisa
New secoiridoid ester of swertiamarin and secoxyloganic acid with hepatoprotective activity from Centaurium spicatum L.
Journal of Pharmacy & Pharmacognosy Research, vol. 3, núm. 3, mayo-junio, 2015, pp. 69-76
Asociación de Académicos de Ciencias Farmacéuticas de Antofagasta
Antofagasta, Chile

© 2015 Journal of Pharmacy & Pharmacognosy Research, 3 (3), 69-76
ISSN 0719-4250
http://jppres.com/jppres

Short Communication | Comunicación Corta

New secoiridoid ester of swertiamarin and secoxyloganic acid with hepatoprotective activity from *Centaurium spicatum* L.

[Nuevo éster secoiridoide de swertiamarin y ácido secoxilogánico de *Centaurium spicatum* L.]

Ahmed E. Allam[1*], Alaa M. Nafady[1], Mohamed A. El-Shanawany[2], Fumide Takano[3], Tomihisa Ohta[3]

[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.
[3]Laboratory of Pharmacognosy and Chemistry of Natural Products, School of Pharmaceutical Sciences, Kanazawa University, Kanazawa 920-1192, Japan.
[*]E-mail: aallam81@yahoo.co.uk

Abstract

Context: Centaurium spicatum (L.) Fritsch (*Gentianaceae*) is an annual herb occurring in Southern Europe and Northern Africa where it is used in traditional medicine for treatment of numerous illness.

Aims: To isolate compounds from *C. spicatum* having hepatoprotective activity.

Methods: Structure elucidation of the new compound was established on the basis of 1D and 2D NMR analyses including ^{1}H-, ^{13}C- NMR, DEPT-135 NMR, HMQC, HMBC and H- H HOHAHA experiments in addition to FAB^{+} and HRFAB^{+} Mass spectrometry. In vivo transaminases inhibition activity was carried out.

Results: A new ester of swertiamarin and secoxyloganic acid (**1**) was isolated from *C. spicatum* in addition to its known components secoiridoids swertiamarin (**2**) and secoxyloganic acid (**3**). All tested secoiridoids have transaminases inhibition activity with different values for each compound **1, 2, 3** (86, 83, 81%) for SGOT and (96, 93, 91%) for SGPT, respectively.

Conclusions: The new ester compound from *C. spicatum* has hepatoprotective activity over than its components.

Keywords: *Centaurium spicatum*; ester of swertiamarin and secoxyloganic acid; hepatoprotective activity; NMR analysis.

Resumen

Contexto: Centaurium spicatum (L.) Fritsch (*Gentianaceae*) es una hierba anual que crece en el sur de Europa y norte de África, donde se utiliza en la medicina tradicional para el tratamiento de numerosas enfermedades.

Objetivos: Aislar compuestos a partir de *C. spicatum* con actividad hepatoprotectora.

Métodos: La elucidación estructural del nuevo compuesto fue establecida sobre las bases del analisis de RMN 1D y 2D que incluyó experimentos de ^{1}H-, ^{13}C- NMR, DEPT-135 NMR, HMQC, HMBC y H- H HOHAHA, además espectrometría de masas FAB^{+} and HRFAB^{+}. También fue medida la actividad de transaminasas in vivo.

Resultados: Un nuevo éster del ácido swertiamarin y ácido secoxilogánico (**1**) de *C. spicatum*, además de compuestos secoiridoides conocidos como swertiamarin (**2**) y ácido secoxilogánico (**3**). Todos los secoididoides tuvieron actividad inhidora sobre transaminasas con diferentes valores para cada compuesto 1, 2, 3 (86, 83, 81%) para SGOT y (96, 93, 91%) para SGPT, respectivamente.

Conclusiones: El nuevo éster de *C. spicatum* tiene actividad hepatoprotectora superior a la de sus componentes.

Palabras Clave: Actividad hepatoprotectora; análisis por RMN; *Centaurium spicatum*; éster de swertiamarin y ácido secoxilogánico.

ARTICLE INFO

Received | Recibido: February 20, 2015.
Received in revised form | Recibido en forma corregida: May 23, 2015.
Accepted | Aceptado: June 7, 2015.
Available Online | Publicado en Línea: June 27, 2015.
Declaration of interests | Declaración de Intereses: The authors declare no conflict of interest.
Funding | Financiación: This work was supported in part by Laboratory of Pharmacognosy and Chemistry of Natural Products, School of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan.
Academic Editor | Editor Académico: Edgar Pastene.

INTRODUCTION

Centaurium spicatum (L.) Fritsch is an annual herb occurring in Southern Europe and Northern Africa where it is used in traditional medicine for treatment of abdominal pain, hypertension, gallstones, kidney and ureter stones, renal colic, wounds and diabetes. A survey of the current literatures revealed the isolation and identification of secoiridoids (sweroside, swertiamarin and gentiopicrin) and polyoxygenated xanthones from the plant (Bishy et al., 1979; Vander and Labadie, 1981; El-Shanawany et al., 1989; Ahmed et al., 2011; El-Shanawany et al., 2011; Ahmed et al., 2012). Alkaloids of the pyridine type (e. g. gentianine), spicatine and the series of amides derived from the secoiridoid glucoside swertiamarin and kantaurin are also shown to be present (Bishy et al., 1979).

In the present work, we report the isolation and structure elucidation of a new ester of swertiamarin and secoxyloganic acid **(1)** together with its known components swertiamarin **(2)** and secoxyloganic acid **(3)**. These compounds were tested for the hepatoprotective activity through reducing the liver microsomal enzymes. The general methodology used for the structure elucidation and the spectral assignments of the new ester glucoside from *C. spicatum* is discussed in this paper.

MATERIAL AND METHODS

Plant material

Aerial parts of *C. spicatum* (L.) Fritsch (*Gentianaceae*) were collected in May 2009 from New Valley, 200 km southwest of Assiut City, Egypt. The plant was identified and authenticated by Prof. A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University. A voucher specimen (CRs-3b = cpd. 1) was deposited at the Department of Chemistry of Natural Products, School of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan.

Extraction and isolation

Air-dried *C. spicatum* aerial parts (4 kg) was extracted thrice with MeOH (5 L of each) at room temperature. The solvents were combined and filtered through filter paper (Advantec MFS Incorporated). The solvent was removed under reduced pressure at 40°C to yield the methanol extract (550 g), which was suspended in 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 hepatoprotective activity 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 with 75% methanol from the *n*-butanol fraction (20.5 g) was further separated by chromatography on ODS column (80 × 200 mm) (Cosmosil 140 C_{18} PREP, Nacalai Tesque, Tokyo, Japan) using six 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 50% CH_3CN (2.6 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 4:1 $CHCl_3$-MeOH was further separated 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 30% CH_3CN in H_2O. This resulted in elution of compound **(1)**.

Spectroscopic methods

NMR spectra were recorded on JOEL EC-600 spectrometer operating at 600.17 MHz for ^{1}H-NMR and 150.92 MHz for ^{13}C-NMR at 20.1°C in CD_3OD (99.5% D) in 5 mm sample tubes. The solvent peaks at δ_H 3.30 in the ^{1}H-NMR spectra and δ_C 49.00 in ^{13}C-NMR spectra, respectively were used as internal references downfield of tetramethylsilane (TMS) at 0 ppm. Spectral widths were 9008 Hz (26 K acquisition points) and 37878 Hz (26 K acquisition points) for ^{1}H- and ^{13}C-NMR, respectively. Chemical shifts are presented in ppm downfield of TMS. For CH_n groups, *n* were determined in DEPT-135 yielding 180° phase difference between -CH_2- signals on the one hand and -CH- and -CH_3- signals on the other. Proton-proton chemical shift correlations were obtained in H- H HOHAHA experiment. Proton-carbon chemical shift correlations were obtained in inversely detected HMQC and HMBC experiments. Fast atom bombardment (FAB) Mass spectra were carried out on MStation instrument in the positive ion mode, using glycerol as the liquid matrix.

Hepatoprotective activity of both the ester and its components isolated from *Centaurium spicatum* L.

In this study, kits utilize the spectrophotometric method of Karmen (Karmen et al., 1955) where 2,4 dinitrophenyl hydrazine is used to convert both oxaloacetate and pyruvate transaminases to the corresponding 2,4-dinitrophenyl hydrazine derivatives, which can be measured spectrophotometrically at 555 nm. The present study was aimed to evaluate the hepatoprotective activity of the compounds **(1-3)** which were isolated from *Centaurium spicatum* on mice liver damage induced by concanavalin A in mice within 8 h through orally dose 1.5 mg/kg body weight (Tiegs et al., 1992) and silymarin in a dose of 50 mg/kg body weight as a positive control (Féhe et al., 2012) (Table 2).

Animals

The study was carried out on male sex C3H rats (19.5 – 21.2 g) (Mahaveer Enterprises, Hyderabad, India). They were allowed to take standard pellet food (National Institute of Nutrition, Hyderabad, India) and water *ad libitum*. Before experiment the rats were kept in standard environmental conditions with room temperature 19 – 25°C relative humidity (55 ± 5%) and 12 h light/12 h dark cycle. All rats received humane care in accordance to the "Guide for the Care and Use of Laboratory Animals" (National Academies Press, Washington, DC, USA, 1996).

Preparation of suspensions

Samples (+ concanavalin A 5 µg/kg bw) and standard silymarin were suspended in distilled water using sodium carboxymethylcellulose (sodium CMC, 0.3%) and administered orally to the animals with the help of an intragastric catheter.

Methodology

The mice were randomly divided into seven groups of five animals each. Group I served as concanavalin A treated control and received the vehicle (sodium CMC 0.3%, 5 mL/kg body weight) + concanavalin A, 5 µg/kg bw. Group II was served as a normal group and received the vehicle (sodium CMC 0.3%, 5 mL/kg body weight). Group III was served with standard drug silymarin at 100 mg/kg body weight. Groups IV, V, VI, and VII were treated with compound **1, 2, 3** at the dose level of 100 mg/kg body weight. All these treatments were given orally for three days. On the last day of the treatment, the animals of groups III–VI received a single dose of concanavalin A in distilled water at 5 µg/kg bw orally after 1 h of the vehicle, samples or standard silymarin treatments. On the 4th day, the animals were anesthetized with anesthetic ether and blood was collected from the abdominal artery and kept for 30 min at 4°C. Serum was separated by centrifugation at 2500 rpm for 15 min at 4°C and used for the biochemical estimations. SGOT and

SGPT were measured spectrophotometrically (Shimadzu 160-A UV-VIS, Koyoto, Japan).

Statistical analysis

Ratio (percentage of control) of liver microsomal enzymes was determined. Data are mean ± SD. Statistical significance was determined by Dunnett's multiple tests after one-way analysis of variance (ANOVA) with a 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

Chemical identification of compound 1-3

Compound **(1)** was obtained as a white amorphous powder (17 mg) with [α] D 20.6 -57.4° (c = 0.2 MeOH). Structure elucidation of the new compound was established on the basis of 1D and 2D NMR analyzes including 1H-, 13C- NMR, DEPT-135 NMR, HMQC, HMBC and H-H. HOHAHA experiments in addition to FAB+ and HRFAB+ Mass spectrometry.

1H- NMR spectrum (600 MHz, CD_3OD) showed the presence of an olefinic signal at δH 7.59 (s, H-3). Furthermore, a doublet at δH 5.68, m was characteristic of an acetalic H-l, and a broad singlet at δH 5.25 (2H, dd, J =2.0, 9.9 Hz, vinylidene group H-10). A signal at δH 1.86 (ddd, J 6α-6β = 13.0, J 6α-7α = 5.5, J 6α -7β = 12.0 Hz, H-6 at α--position) while that of β-position was shown at δH 1.71 (m, H-6 at β-position). The protons of H-7 (α and β) were represented by the signals at δH 4.31 (ddd, J 7α -6α = 2.2, J 7α -6β = 2.2, J 7α -6β = 11.3 Hz, H-7α) and at δH 4.71 (dd, J 7β -6α = 10.9, J 7β-6α = 2.7 Hz, H-7 β). The signal at δH 2.87 (d, J = 9.9 Hz) was assigned to H-9. 13C-NMR spectrum of this part of the compound displayed 16 signals. Out of these signals, six signals were assigned to glucose moiety while the rest of them was assigned to the aglycon portion, on the basis of similar values reported for sweroside (Bianco, 1994).

The chemical structure difference between sweroside and swertiamarin is the presence of the hydroxy group at C-5 in swertiamarin (Cornelis and Chapelle, 1976; Popove et al., 1987) and this is the alcoholic part of the ester (Table 1).

For the acidic part, 1H-NMR spectrum showed the presence of an olefinic signal at δH 7.44 (s, H-3), furthermore, a doublet at δH 5.42, m was characteristic of an acetalic H-l whereas the signal at δH 5.18 (2H, dd, J = 2.0, 9.9 Hz, H-10) was indicative of the presence of a vinylidene group. A signal at δH 2.20, m was assigned to the proton of C-6 exist at α-position while the proton present at β-position showed a signal at δH 3.0 (dd, J 6β-6α = 16.8 Hz, J 6β-H-5 = 4.4 Hz). The spectrum also showed a signal at δH 2.92 (d, J = 9.6 Hz, H-9) and a signal at δH 3.30 (3H, s, H-11-OCH_3). 13C-NMR spectrum of that part displayed 17 signals. Out of them, six signals were assigned to glucose moiety while the rest of them was assigned to the aglycon portion. From the previous data, the acidic part could be simply identified as secoxyloganic acid part comparing with the literature (Ghosal et al., 1974; Munir et al., 1996).

The linkage between the two parts could be confirmed from HMBC experiment where a correlation between H-5 of the secologanic acid (δH 3.25, m) and the carbonyl ester δc 176.3 adjacent to C-5 of the swertiamarin moiety was observed. Also the correlations between protons of both moieties in H-H.HOHAHA experiment is supporting the ester linkage between them.

Also, acid hydrolysis of the ester was carried out and resulted in the presence of both moieties swertiamarin and the secoxyloganic acid that was identified by 1H - and 13C- NMR.

The molecular weight of compound **(1)** was 760.24 with a molecular formula $C_{33}H_{44}O_{20}$ that was evident from FAB+ [M+H] at 761.0 and HRFAB+ Mass at m/z 761.2503.

All the previous data assure that compound **(1)** is composed of an ester of both swertiamarin **(2)** and secoxyloganic acid **(3)** which is a new compound (Table 1, Fig. 1).

Table 1. ^{13}C- and ^{1}H-NMR assignments for compound **(1)** recorded in CD_3OD.

Position	**^{13}C-NMR, (δ, mult.)**	**^{1}H-NMR, [δ, mult, *J* (Hz)]**
1	99.0,d	5.68,m
3	154.6,d	7.59,s
4	108.8,s	-
5	64.2,s	-
6	33.6,t	*α*: 1.86, ddd, 5.1,13.0,12.0 *β*: 1.71, m
7	65.9,t	*α*: 4.31,ddd, 1.7, 5.1, 10.9 *β*: 4.71,dd, 2.7, 10.9
8	134.5,d	5.66.m
9	51.9,d	2.87, d, 9.9
10	121.2,t	5.25, dd, 2.0, 9.9
11	167.9,s	-
Gluc.		
1'	99.8,d	4.62,d,7.9
2'	74.4,d	3.65-4.33,m
3'	77.7,d	3.65-4.33,m
4'	71.3,d	3.65-4.33,m
5'	78.3,d	3.65-4.33,m
6'	62.5,t	a: 3.85,dd,2.7,12.0 b: 3.65,m
1''	97.4,d	5.42,m
3''	153.4,d	7.44,s
4''	110.1,s	-
5''	28.3,d	3.25,m
6''	34.8,t	*α*: 2.20,m *β*: 3.0,dd,16.8, 4.4(H6*α*-H6*β*, H6*β*- H5)
7'	176.3,s	-
8'	133.7,d	5.35,m
9'	45.2,d	2.78,m
10'	120.4,t	5.18,dd, 2.0, 9.9
11'	170.2,s	-
$COOCH_3$	51.9,q	3.30,3H,s
Gluc.		
1''	100.2,d	4.60,d,7.9
2''	74.6,d	3.65-4.33,m
3''	77.9,d	3.65-4.33,m
4''	71.5,d	3.65-4.33,m
5''	78.5,d	3.65-4.33,m
6''	62.6,t	a: 3.62,dd,2.7,12.0 b: 3.65,m

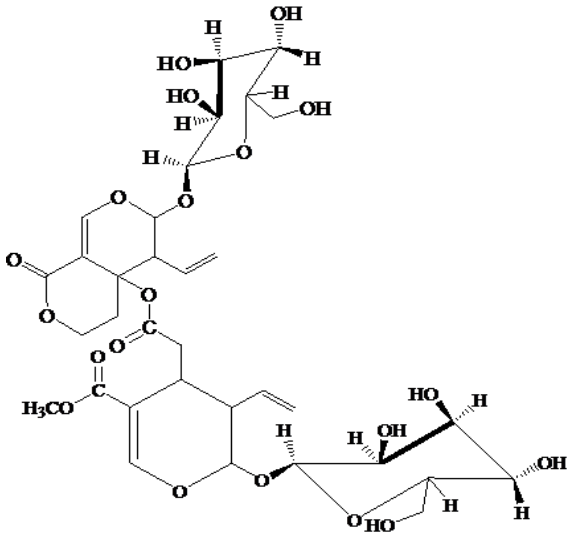

Figure 1. Compound (**1**), ester of swertiamarin and secoxyloganic acid.

Hepatoprotective effect of secoiridoids (compounds 1-3) isolated from *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 (Watson et al., 1999; Kubes et al., 2000).

It is noted (Table 2) that the all tested secoiridoids inhibited transaminase activities with different values for each compound **1, 2, 3** (86, 83, and 81%) for SGOT and (96, 93, and 91%) for SGPT, respectively which means that the new ester compound has hepatoprotective activity over than its components.

In recent studies, swertiamarin was isolated from successive ethyl acetate extract of the plant *Enicostemma axillare* (*Gentianaceae*). The concentration of swertiamarin was determined by high performance thin layer chromatography. The hepatoprotective and antioxidant activity of swertiamarin was carried out against d-Galactosamine (d-GalN) (200 mg/kg body weight, i.p.) induced liver injury in rats. The treatment with swertiamarin at 100 and 200 mg/kg body weight when administered orally for 8 days prior to d-GalN caused a significant restoration of all the altered biochemical parameters due to d-GalN towards the normal, indicating the potent antioxidant and hepatoprotective nature of swertiamarin (Jaishree and Badami, 2010).

CONCLUSIONS

The present study evaluates the hepato-protective activity of compounds **(1-3)** isolated from *Centaurium spicatum* on mice liver damage induced by Concanavalin A in mice within 8 h through a dose 1.5 mg/kg body weight and silymarin in a dose of 50 mg/kg body weight as a positive control and noted (Table 2) that all tested secoiridoids have transaminases inhibition activity with different values for each compound **1, 2, 3** (86, 83, 81%) for SGOT and (96, 93, 91%) for SGPT, respectively which means that the new ester compound has hepatoprotective activity over than its components.

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

Parameters	**SGOT** (IU/L)	**SGPT** (IU/L)
Normal	46.9 ± 5.40	45.3 ± 4.25
Concanavalin A	428.2 ± 2.52*	520.1 ± 3.63*
Silymarin + Concanavalin A	80.2 ± 2.32*	51.2 ± 2.36*
Compound **1** + Concanavalin A	72.3 ± 5.42*	35.2 ± 1.57*
Compound **2** + Concanavalin A	67.0 ± 3.50*	25.5 ± 4.16*
Compound **3** + Concanavalin A	57.1 ± 2.50*	48.5 ± 1.72*

Concanavalin A (1.5 mg/kg bw, intravenous), silymarin (50 mg/kg bw. via) and compounds 1-3 (100 mg/kg bw, intragastrically). Normal group: This group received the vehicle (sodium CMC 0.3%, 5 mL/kg body weight). Each group represents the responses of five animals per group as mean ± SD. *p < 0.05 represents the statistical difference between treated group and control (vehicle).

Electronic supplementary material

Spectroscopic data of compounds **1, 2** and **3** are available as Electronic supplementary material. The online version of this article contains supplementary material, which is available to authorized users.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

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

REFERENCES

Ahmed EA, El-Shanawany MA, Enaam YB, Alaa MN, Fumihide T, Ohta T (2011) Two new acetylated flavonoid glycosides from *Centaurium spicatum* L. J Nat Med 66(2): 388-393.

Ahmed EA, El-Shanawany MA, Enaam YB, Alaa MN, Fumihide T, Ohta T (2012) Cytotoxic xanthon glycosides from aerial part of *Centaurium spicatum*. Heterocycles 85(3): 651- 659.

Bianco A (1994) Recent developments in iridoids chemistry. Pure Appl Chem 66: 2335-2338.

Bishay DW, Ross SA, Hylands PJ (1979) Alkaloids and glycosides from *Centaurium spicatum*. Planta Med 37: 253-258.

Cornelis A, Chapella JP (1976) Apport de la re'sonance magnetique nucleaire du carbone 13 (NMR 13C) a l'etude systematique des glucosides seco-iridoides: confirmation de structure du swertiamaroside. Pharm Acta Helv 51: 177-180.

EL-Shanawany MA, Makboul MA, Abdel Baky AM (1989) Phytochemical study of *Centaurium pulchellum* (SW) Druce growing in Egypt. Bull Pharm Sci Assiut Univ 12: 416–421.

EL-Shanawany MA, Samir AR, Gamal AM, Alaa MN, Sabrin RM (2011) A new xanthone from the roots of *Centaurium spicatum* L. Phytochem Lett 4: 126-128.

Féher J, Lengyel G (2012) Silymarin in the prevention and treatment of liver diseases and primary liver cancer. Curr Pharm Biotechnol 13(1): 210-217.

Ghosal S, Singh AK, Sharma PV, Chaudhuri RK (1974) Chemical constituents of gentianaceae. IX. Natural occurrence of erythrocentaurin in *Enicostemma hyssopifolium* and *Swertia lawii*. J Pharm Sci 63(6): 944-945.

Jaishree V, Badami S (2010) Antioxidant and hepato-protective effect of swertiamarin from *Enicostemma axillare* against d-galactosamine induced acute liver damage in rats. J Ethnopharmacol 130(1): 103-106.

Karmen A, Blewski F, LaDue J (1955) Transaminase activity in human blood. J Clin Invest 34: 126-131.

Kubes P, McCafferty D (2000) Nitric oxide and intestinal inflammation. Am J Med 109: 150–158.

Munir A, Mansoor A, Muhammad A, Khalid A (1996) *Enicostema littorale*: A new source of swertiamarin. Pak J Pharm Sci 9(l): 29-35.

Popov V, Supin A (1987) Hearing characteristic of the white whale (in Russ.) Dokl. Akad. Nauk SSSR. Proc Acad Sci USSR 294: 1255-1258.

Tiegs G, Hentschel J, Wendel A (1992) A T cell-dependent experimental liver injury in mice inducible by concanavalin A. J Clin Invest 99(1): 196–203.

Vander SWG, Labadie RP (1981) Secoiridoids and xanthones in the genus *Centaurium*. Part III: Decentapicrins A, B and C, new m-hydroxybenzoyl esters of sweroside from *Centaurium littorale*. Planta Med 4: 150-160.

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