Analgesic effect of *Centaurium erythraea* and molecular docking investigation of the major component swertiamarin

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ABSTRACT

Centaurium erythraea Rafn is employed in the Algerian traditional medicine for treating pain. The analgesic activity of the ethanolic extract (EE) from the flowering aerial parts of this plant was examined and molecular docking of the main bioactive compound was performed. The EE, characterized by the iridoid swertiamarin, was administered to Wistar albino rats in pain models. Peripheral analgesic activity was evaluated using the acetic acid-induced writhing test and hot plate test was performed for central anti-nociceptive activity evaluation. Treatment with EE significantly decreased rats' writhing induced by acetic acid suggesting peripheral analgesic activity. Furthermore, the elevation of mean basal reaction time in the hot plate method indicated the central analgesic activity. Molecular docking studies showed good docking energy with acceptable binding interactions of swertiamarin with cyclooxygenase-2 protein. This supports the analgesic activity of *C. erythraea* EE, justifying the traditional use of the plant as analgesic herbal remedy.

Experimental

Plant material and extraction

The flowering aerial parts of *C. erythraea* were collected from M'sila at 35°42' 21"N and 4°32'31" E (Algeria). The plant was identified and taxonomically authenticated by D. Sarri Dj. (Department of Nature Sciences and Life, University of M'sila). A voucher specimen of the plant (AB-35) was deposited in the herbarium of the same Department. The plant material was rinsed, dried in the shade at room temperature, and finely ground to a powder. The ethanolic extract (EE) was prepared by using a Soxhlet apparatus. Fifty g of plant powder were extracted with 500 mL of ethanol for 6 h (Bimakr et al. 2011). The extract was filtered and evaporated under reduced pressure by a rotary evaporator (Buchi R-210).

Animals

Wistar albino rats (180 – 200 g) were obtained from Pasteur Institute Algiers (Algeria). They were fed ad libitum with a water and kibble diet. All experimental protocols were in accordance with the European Community Council Directive (86/609/EEC) and approved by the National Committee for Evaluation and Programming of University Research of Algerian Ministry of Higher Education and Scientific Research (Registration N°: DO1N01UN280120150001).

HPLC-PDA-MS analysis

General chromatographic procedures

Medium-pressure liquid chromatography (MPLC) was performed on a Biotage® Selekt Flash Purification System controlled with SELEKT 1.3-10998 software (Biotage, Sweden). The sample was prepared as a liquid load in DMSO and directly injected onto the column. A pre-packed C18 RediSep Rf Gold® High Performance cartridge (100 g, HP C18, column volume (CV) 87.7 mL, Teledyne Isco, USA) was used for MPLC. The flow rate was 60 mL/min. Preparative HPLC was carried out on a Preparative LC/MSD System (Agilent Technologies, Santa Clara, CA, USA)

consisting of a quaternary pump (1200 Series, 1290 Infinity II 1260 Prep Bin Pump), a PDA detector (1100 Series), and a 6120 Quadrupole LC/MS. A SunFire Prep C18 OBD column (5 µm, 30 x 150 mm i.d., Waters, Milford, MA, USA), equipped with a C18 Prep Guard Cartridge (10 x 30 mm i.d.) was used. The flow rate was 20 mL/min. Data acquisition and processing was performed using ChemStation software (Agilent Technologies). For injection a 1290 Infinity II 1290 Valve Drive manual injection system (Agilent Technologies) was used. Ultrapure water was obtained from a Milli-Q water purification system (Merck Millipore, Darmstadt, Germany). HPLC-grade acetonitrile was purchased from Avantor Performance Materials (Radnor Township, PA, USA).

HPLC-UV-ELSD-MS analysis

HPLC-UV-ELSD-MS analysis was carried out on a chromatographic system consisting of a degasser, quaternary pump (LC-20AD), a column oven (CTO-20AC), a PDA detector (SPD-M20A) (Shimadzu, Kyoto, Japan), an evaporative light scattering detector (Alltech 3300 ELSD) (Büchi Labortechnik, Flawil, Switzerland), and a triple quadrupole mass spectrometer (LCMS-8030) (Shimadzu). Separation was performed on a SunFire C18 column (3.5 µm, 3.0 x 150 mm i.d., Waters) equipped with a guard column (3.0 x 10 mm). Water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B) were used as mobile phase. A gradient of 5-80% B in 30 min followed by 80-100% B in 5 min was applied at a flow rate of 0.4 mL/min. The extract was dissolved in DMSO at a concentration of 10 mg/mL and 10 µL were injected. The LabSolutions software (Shimadzu) was used for data acquisition and processing.

Isolation and characterization of dominant compound, swertiamarin

The EE of *C. erythraea* (2 g) was dissolved in 4 mL DMSO and then fractionated by MPLC on a C18 RediSep Rf Gold® High Performance cartridge with a gradient of acetonitrile in water (both containing 0.1% formic acid): MeCN 5% for 1 CV, 5-9% for 3.5 CV, 9-40% for 3.3 CV, 40-77% for 1.2 CV, 77% for 3.4 CV, at a flow rate of 60 mL/min. In total, 7 fractions (F1-F7) were collected

based on UV-detection at 254 nm. Further purification of Fraction F2 (218 mg) by preparative HPLC with a gradient of 5–80% acetonitrile in water (both containing 0.1% formic acid) in 30 min afforded the main compound, swertiamarin (122.4 mg, t_R= 9.7 min, purity ca. 95%). NMR spectra were recorded on a Bruker Avance III spectrometer (Rheinstetten, Germany) operating at 500.13 MHz for ^{1}H. ^{1}H NMR and 2D NMR spectra were measured in DMSO-d6 (ARMAR Chemicals) with a 1 mm TXI probe at 23°C. Data were analyzed using Topspin (Bruker) and Spectrus Processor (ACD/Lab, Toronto, Canada) softwares.

Evaluation of analgesic activity

Acetic acid-induced writhing test

Animals were divided into 5 groups of 5 rats each as follows: group 1, negative control, received distilled water; groups 2, 3 and 4 received 100, 200 and 300 mg/kg b.wt. of EE, respectively; and group 5, the positive control, received paracetamol (Sanofi, Paris, France) 150 mg/ kg b.wt, as standard drug. After 30 min, writhing was induced in rats by intraperitoneal injection of 0.6% acetic acid (10 mL/kg b. wt), and the number of writhing was counted over a period of 20 min (Koster 1959). The percentage of writhing inhibition was calculated using the following equation (Saleem et al. 2011):

$$\text{Inhibition (\%)} = \frac{\text{Mean N°. of writhing (control)} - \text{Mean N°. of writhing (test)}}{\text{Mean N°. of writhing (control)}} \times 100$$

Hot plate test

Animals were divided into 5 groups of five rats each as follows: group 1, negative control, received distilled water; groups 2, 3, and 4 received 100, 200, and 300 mg/kg b.wt of EE, respectively; and group 5, the positive control, received paracetamol 150 mg/kg b.wt, as standard drug. Rats from each group were placed on a hot plate (55±0.5^o C). The latency time for paw licking or jumping was taken

as reaction time. The reaction time was recorded at 30, 60, and 90 min following the different treatments (Yaya Soro et al. 2009).

Molecular docking studies

The crystal structure of COX-2, PDB ID 6COX, corresponding to COX-2 protein complexed with its selective inhibitor SC-558, was imported into the AutoDockTools program (Drwal et al. 2014). The ligand and cofactors were removed, and all missing hydrogens were added. In addition, the active site was taken to be 8 Å from the center of the crystal ligand (Dionne et al. 2001). The Gasteiger charges were assigned, and non-polar hydrogens were merged with their corresponding carbons (Morris et al. 2009).

The structure of swertiamarin, was drawn with Avogadro software (Bouaziz-Terrachet et al 2013; http://avogadro.cc/), where geometrical optimization was performed with Ghemical force field.

The optimized geometry of swertiamarin was imported in AutoDockTools and flexibly docked into the active site of COX-2. A grid map of 56×50×60 points around the modeled domain was used with a grid point spacing of 0.375 Å. The Lamarckian genetic algorithm (LGA), implemented in Autodock 4 software (Hanwell et al. 2012), with default docking parameters, was used to predict binding mode, binding energy, and orientation of extracted compound at the active site of the selected protein. At the end of docking, the best conformation of the ligand was analyzed for its binding interactions.

Statistical analysis

The results are expressed as the mean ± standard deviation. Data were analyzed using the GRAPH PAD (version 7). Differences between groups were assessed by Tukey's test. Differences were considered significant at $p < 0.05$.

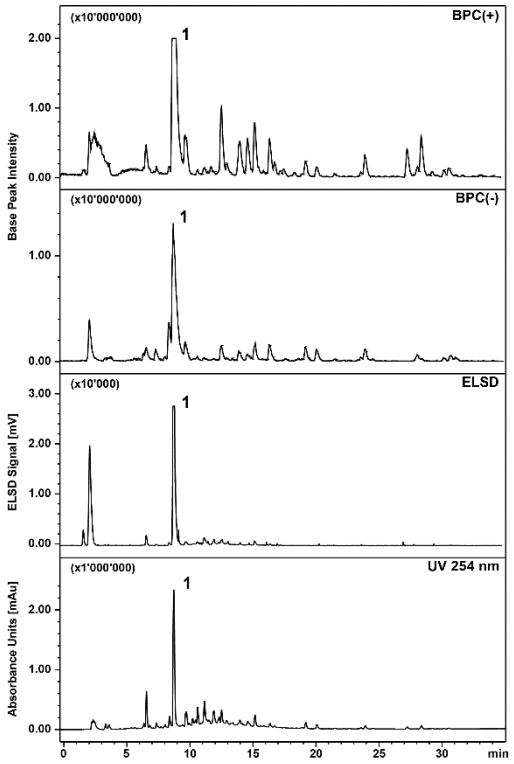

Figure S1. HPLC-PDA-ELSD-ESIMS chromatogram of EE extract of *C. erythraea* (C18 column, 5–80% acetonitrile in 30 min). Peak 1 corresponds to swertiamarin. BPC: Base peak chromatogram. ELSD: Evaporative light scattering detection.

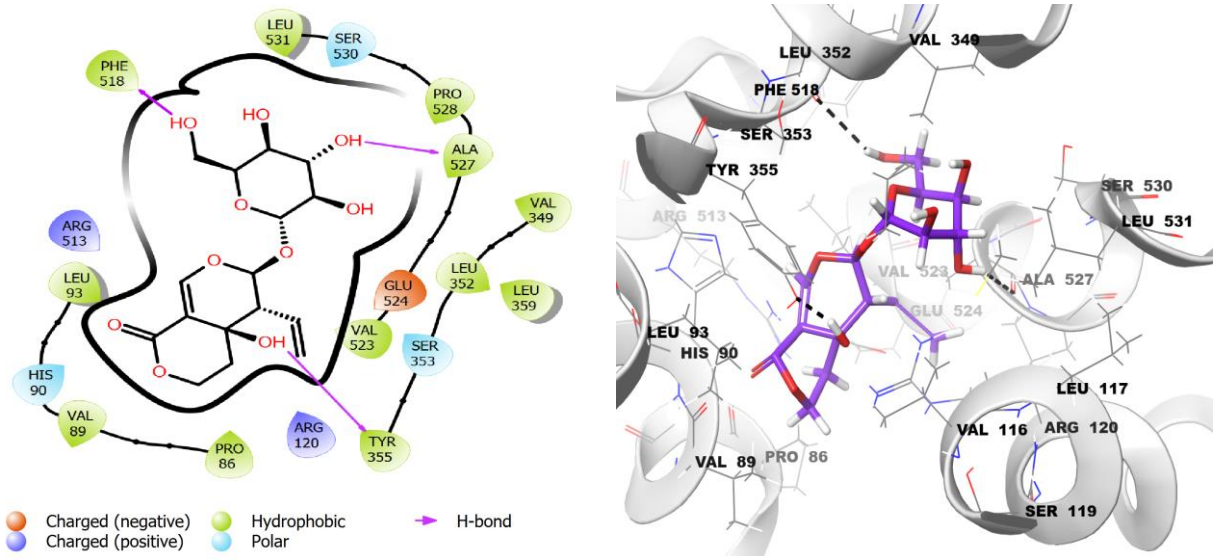

Figure S2. 2D and 3D views of the docked pose of swertiamarin in the active site of COX-2 protein.

Table S1. ^{1}H and ^{13}C NMR Spectroscopic data for swertiamarin (DMSO-d6; 500.13 Hz; δ in ppm).

		Swertiamarin
Position	δ_C	δ_H (mult *J* in Hz)
1	96.4, CH	5.59 d (1.2)
2	151.9, CH	7.51 s
3	107.9, C	-
4	164.1, C	-
5	64.0, CH_2	4.59 m 4.27 m
6	31.9, CH_2	1.71 m
7	62.1, C	-
7-OH	-	3.34 br s
8	49.8, CH	2.84 br dd
9	132.7, CH	5.36 m [a]
10	120.3, CH_2	5.25 m 5.38 m [a]
1’	98.1, CH	4.47 d (7.6)
2’	72.7, CH	3.00 t (8.39, 8.4)[a]
2’-OH	-	4.88 br s [a]
3’	76.0, CH	3.19 m [a]
3’-OH	-	4.88 br s [a]
4’	69.9, CH	3.08 t (8.85, 8.8)
4’-OH	-	4.68 br s [a]
5’	77.3, CH	3.18 m [a]
6’	60.8, CH_2	3.46 dd (5.19, 6.4) 3.69 dd (11.90, 1.8)
6’-OH	-	4.68 br s [a]

Notes: assignments based on COSY, HSQC, and HMBC data. ^{13}C NMR data extracted from HSQC and HMBC spectra.

[a] Overlapping signals.

Table S2. Analgesic effects of *C. erythraea* EE on acetic-acid-induced writhing in rats.

Groups	Dose (mg/kg)	Average number of writhes	% Inhibition
Control	-	307.2 ± 15.1	-
EE	100	155.2 ± 12.0***	49.5
	200	103.6 ± 12.9***	66.3
	300	81.2 ± 16.7***	73.6
Paracetamol	150	167.8 ± 6.9***	45.3

Notes: values are expressed as mean ± SD (n = 5).
*** $p < 0.001$ when treated groups are compared to the negative control group.

Table S3. Analgesic effects of *C. erythraea* EE in hot plate test in rats.

Groups	Dose (mg/kg)	Reaction time in seconds at various time intervals			
		0 min	30 min	60 min	90 min
Control	-	5.0 ± 0.1	5.0 ± 0.1	5.3 ± 0.5	6.0 ± 0.0
EE	100	5.6 ± 0.5	6.0 ± 0.0*	8.0 ± 0.3**	9.0 ± 0.6**
	200	5.3 ± 0.1	6.5 ± 0.1*	7.6 ± 0.2**	9.3 ± 0.0**
	300	5.3 ± 0.1	7.0 ± 0.3**	11.3 ± 0.5***	15.3 ± 0.5***
Paracetamol	150	5.6 ± 0.0	6.5 ± 1.0*	10.0 ± 0.3***	11.3 ± 0.6***

Notes: values are expressed as mean ± SD (n = 5).
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when treated groups are compared to the negative control group.

Table S4. Hydrogen bonds, interaction with positively charged and polar residues, predicted for swertiamarin, with cyclooxygenase-2 protein.

Interactions	Swertiamarin
H-Bond	Tyr355 (2.1 Å), Ala527 (2.7 Å), Phe518 (2.5 Å)
Hydrophobic	Phe518, Leu531, Pro528, Ala527, Val523, Val349, Leu352, Leu359, Tyr355, Pro86, Val89, Leu93
Charged (positive)	Arg513, Arg120, Glu524
Polar	Ser530, Ser353, His90

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