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Grandivittin as a natural minor groove binder extracted from *Ferulago macrocarpa* to ct-DNA, experimental and in silico analysis

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ABSTRACT

Ferulago macrocarpa (Fenzl) Boiss., is an endemic medicinal herb of Iran. In this study a dihydrofuranocoumarin called grandivittin (GRA) was separate and purified from *Ferulago macrocarpa* (Fenzl) Boiss, and characterized by ^{1}H NMR and Mass spectroscopic methods. The electrochemical behavior of GRA was evaluated by cyclic voltammetry (CV). The interaction of GRA with calf thymus double strand deoxyribonucleic acid (ct-DNA), was evaluated by CV, differential pulse voltammetry (DPV), fluorescence, UV–Vis, FT-IR and molecular modeling methods. The thermodynamic parameters of GRA-DNA complex were measured and reported as: $\Delta H = 15.04$ kJ mol^{-1}, $\Delta S = 105.54$ J mol^{-1} and $\Delta G = -15.62$ kJ mol^{-1}. Docking simulation was performed to investigate the probable binding mode of GRA to various DNA, too. The polymerase extension study was performed using real-time PCR to confirm the inhibitory effect of GRA on polymerase extension activity as a mirror of binding to ct-DNA. However, all data showed that the grooves binding especially minor groove between GRA and ct-DNA is more predominant rather than other binding modes.

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1. Introduction

The inhibition of DNA replication is still considered as a strategy to treatment of bacterial and cancers diseases [1–3]. In this way, the demands for using of natural compounds as antibiotic, antiviral and anticancer agents with fewer side effects caused many researcher were attention to the isolation, identification and biological evaluation of natural products [4–7]. An important way to assess the therapeutic properties of natural compounds is the evaluation of their DNA binding abilities for designing of new drugs [8]. Therefore, the DNA binding of various natural compounds such as morin [9], quercitin [10,11], kaempferide [11], luteolin [11], resveratrol [12], genistein [13] hesperitin [14], naringenin [14], and rutin [15] were studied by spectroscopic and voltammetric methods. Coumarins are a group of phenolics with numerous pharmacological effects especially cytotoxic and antiviral properties [16,17]. Dihydrofurano coumarins are a group of coumarins which are highly active as anticancer [18,19] and cancer preventer [20] agents.

Recently, Ben-Salem et al., reported a remarkable cytotoxic activity for dihydrofuranocoumarins which extracted from *Ferula lutea* (Poir.) Maire with IC_{50} values of 0.29 ± 0.05 and 1.61 ± 0.57 mol L^{-1} against the cell lines of HT-29 and HCT 116, respectively [21]. Additionally, Abad et al., reported the inhibition effects of several dihydrofuranocoumarins on some macrophage functions involved in the inflammatory process [22].

We have purified dihyrocoumarins from the medicinal plant of *Ferulago angulata*, that wildly grown in western Asia [23]. The *Ferulago angulata* popularly is used as anti-inflammatory and food flavoring, and its essential oil has shown larvicidal activity [24] as well. The essential oil and organic solvent extracts of *Ferulago* species have shown antimicrobial [25–29], antioxidant [26,30–32], and antifungal activities [33], too. In addition, several species of *Ferulago* have the cytotoxic and proapoptotic properties. *Ferulago angulata*, demonstrated a time- and dose-dependent inhibition on the proliferation of non-Hodgkin's B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), and human acute myelocytic leukemia (KG-1A) cell lines [34]. Rosselli et al., isolated and

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identified four natural coumarins from roots of *Ferulagocampestris*. They demonstrated that some of these coumarins have marginally cytotoxic effects against the A549 lung cancer cell line [35]. In 2006, Amirghofran and coworkers reported a remarkable inhibition activity for methanolic extract of *Ferulago angulata* on Jurkat cells proliferation. They found out that at concentrations of nearly 180 μg ml^{-1}, a fifty percent inhibition of Fen bladder cell carcinoma was achieved [36]. The previous phytochemical studies on the *Ferulago* species demonstrated that the coumarins, dihydrofuranocoumarins and etc., were made the most constituents of the extracts [28,31,35,37–39].

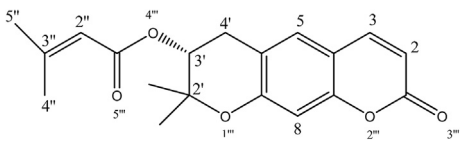
Ferulago macrocarpa (Fenzl) Boiss., is a perennial herb that grows in the west of Iran [40]. The plant popularly referred as Chavile-Roshanball in Persian and its essential oil has shown larvicidal activity [24]. In our laboratory research we could extract and identified several dihydrofuranocoumarins specially grandivittin (GRA) from *Ferulago macrocarpa* (Fenzl) Boiss., (Scheme 1). Previously the antibacterial [41] and cytotoxic effect against the A549 lung cancer cell line [35] of GRA was reported. In addition, the antioxidant activity of the GRA was evaluated by its effect on human whole blood leukocytes and on isolated polymorpho nucleated chemiluminescence [41].

Understanding that how GRA bind at structure of DNA may be used as an important point for elucidating of mechanism of antibacterial, cytotoxic and inhibition of macrophage functions. In this work, the electrochemical behavior of GRA was studied. In addition, the GRA-DNA interaction was investigated in HCl-Tris aqueous buffer solution at the physiological pH 7.3, using voltammetry and multi spectroscopic (Ultraviolet–Visible absorbance (UV–Vis), Fourier transform infrared (FT-IR), and fluorescence) methods. Additionally, the measured thermodynamic parameters were used to interpret the mode of interaction. The effect of GRA on the conformation of DNA, were also interpreted by molecular modeling. Finally we studied the effect of GRA on polymerase extension activity to elucidate blockage of polymerase as an indirect result of binding to DNA. This study clarifies that the biological activity of GRA is due to interaction with DNA and therefore our results demonstrated the details of GRA-DNA complex formation and provided a concept basis for the possible cytotoxic mechanism of dihydrofuranocoumarins.

2. Experimental section

2.1. Chemicals

The calf thymus double strand deoxyribonucleic acid (ct-DNA) was prepared from Sigma Chemical Co., (USA). The high analytical organic solvents such as methanol (MeOH), acetonitrile (AN), ethanol (EtOH), ethylacetate (EtOAc), and n-heptane (Hep), were purchased from Merck (Darmstadt Germany). Double distilled water was used through all the experimental. All other chemicals were purchased from Merck (Darmstadt Germany). The stock solution of ct-DNA was prepared as follows: 2 mg of ct-DNA was dissolved in 4 ml of Tris-HCl buffer (pH 7.3) and dialyzed exhaustively against the same buffer for 24 h [42]. The final solution gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that ct-DNA was sufficiently free from protein.



Scheme 1. The structure of grandivittin (GRA).

2.2. Polymerase extension template

The Bovine thymus satellite I (a part of calf thymus DNA) sequence was used as the extension template. We picked up best optimized primers designed by gene runner 3.05 software. The primers were ordered from Macrogen. The forward primer sequence was TGGGTTTGGTGCATTGGAAG and the reverse primer sequence was AGGTTCCAAAGACAGCTCGA with the product size of 205 nucleotides. The GC content was 50% and primers were negative for hairpin formation.

2.3. Plant preparation

The fruits of wild-growing *Ferulago macrocarpa*, (Fenzl) Boiss., were collected in May 2013 from the Saleh-Abad region (Ilam province, Iran). The taxonomic identification of plant materials was confirmed by Ilam Agricultural and Natural Resource Research Center. The fruits were dried in the shade (at room temperature).

2.4. Instrumental

The spectrophotometric titrations of GRA with ct-DNA were carried out by an HP Agilent (8453) UV–Vis spectrophotometer equipped with a Peltier (Agilent 89090A). A Beckman spectrofluorometer (LS45) was used for fluorescence titration with a maximum excitation wavelength of 332 nm. The pH values of solutions were measured by a Metrohm pH meter model 827.

A voltammeter VA 797 Computrace (Metrohm, Switzerland) was used for all electrochemical experiments. The cyclic voltammetric (CV) and differential pulse voltammetric (DPV) titrations were carried out at a hanging mercury drop electrode in voltammetric cell contain an Ag/AgCl as reference electrode (3 mol L^{-1} KCl), and a platinum wire as an auxiliary electrode. 10 ml of 0.01 mol L^{-1} Tris-HCl buffer solution (pH 7.3) was used as supporting electrolyte which deaerated with pure N_2 gas for 2 min. The FT-IR measurement was carried out by a Shimadzu (IR Prestige-21) equipped with a KBr beam splitter.

Polymerase extension reactions were carried out by using of an StepOne™ Real-Time PCR System (Thermo Fisher Scientific).

2.5. Extraction procedure

The air dried fruits of *F. macrocarpa* (500 g) were extracted with acetone at room temperature. After remove of solvent by vacuum, the residue (87.5 g) was dissolved in methanol, kept in −20 °C and underwent chill filtration to get rid of triglycerides. Defatted extract (75 g) was purified by vacuum liquid chromatography using mixtures of Hep: EtOAc (100:0 to 0:100% v/v) to afford 7 fractions (A–G). Fraction D (Hep: EtOAc, 80:20 v/v) rendered a mass of impure crystals in which recrystallization resulted in pure GRA.

2.6. GRA-DNA interaction procedures

2.6.1. CV titrations of GRA with ct-DNA

All CV titration studies were performed in a constant concentration of the GRA (5.0×10^{-5} mol L^{-1}, 10 ml of electrolyte solution), while the ct-DNA concentration varied ($0.0–2.0 \times 10^{-4}$ mol L^{-1}; r_i = [DNA]/[GRA] = 4). The titrations were performed in the potential range of 0.0 V and a vertex potential of −1.9 V at a given scan rate 80 mV s^{-1}.

2.6.2. DPV titrations of GRA with ct-DNA

All differential pulse voltammograms were recorded in the potential range of 0.0 V to −1.9 V. The DPV titrations were performed at a constant concentration of the GRA (5.0×10^{-5} mol L^{-1}) and

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