Chemical Constituents of *Cymbocarpum erythraeum* (DC.) Boiss., and Evaluation of Its Anti-*Helicobacter pylori* Activity

*Cymbocarpum erythraeum* (DC.) Boiss.’ın Kimyasal Bileşikleri ve *Helicobacter pylori*’ye Karşı Etkisi

**ABSTRACT**

Objective: *Cymbocarpum erythraeum* (Apiaceae) is an endemic species in Iran. Up to now, there have been no phytochemical and biological investigations on this species. Therefore, isolation of the main secondary metabolites of the plant along with its anti-*H. pylori* activity have been considered in this paper.

Materials and Methods: The dried parts of the plant were extracted with different solvents using solvent percolation and the antibacterial activity of the extracts evaluated by the disk diffusion method. Four compounds were isolated using different column chromatography methods.

Results: The compounds were identified by proton nuclear magnetic resonance and carbon-13 nuclear magnetic resonance as isoquercetin (1), rutin (2), β-sitosterol (3) and 2-decenol (4).

Conclusion: Anti-*H. pylori* evaluation of the extracts and isolated compounds against three clinical isolates of *H. pylori* revealed that hexane extract of the plant inhibited all *H. pylori* strains.

Key words: Apiaceae, *C. erythraeum*, chromatography, disk diffusion, *H. pylori*

**ÖZ**

Amaç: *Cymbocarpum erythraeum* (Apiaceae) İran’a endemik bir türdür. Bu zamana kadar, bu tür üzerinde herhangi bir fitokimyasal ve biyolojik aktivite çalışmasına rastlanmamıştır. Bu nedenle, bu çalışmada, bitkinin ana sekonder metabolitlerinin ve *Helicobacter pylori*’ye karşı etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Kurutulmuş bitki kısımları perkolasyon yöntemi ile farklı çözücüler kullanılarak ekstre edilmiş ve ekstrelerin antibakteriyel etkisi disk difüzyon yöntemiyle değerlendirilmiştir. Farklı kolon kromatografisi yöntemleri kullanılarak dört bileşik izole edilmiştir.

Bulgular: Bileşiklerin yapıları, proton nükleer manyetik rezonans ve karbon-13 nükleer manyetik rezonans as isoquercetin (1), rutin (2), β-sitosterol (3) ve 2-dekenol (4) olarak belirlenmiştir.

Sonuç: Ekstrelerin ve izole edilen bileşiklerin üç *H. pylori* klinik izolatına karşı etkilerinin değerlendirildiği bu çalışmada, bitkinin hekzan ekstresinin tüm *H. pylori* suşlarını ihtiba ettiği tespit edilmiştir.

Anahtar kelimeler: Apiaceae, *C. erythraeum*, kromatografi, disk difüzyon, *H. pylori*
INTRODUCTION

Cymbocarpum is represented in Iran by three species that occur naturally in the wild, including Cymbocarpum marginatum, Cymbocarpum erythraeum, and Cymbocarpum anethoides. The essential oils had been obtained from these three species of Cymbocarpum from Iran and analyzed indicating that the oils of all three plants were rich in aliphatic aldehydes. Furthermore, the oil of C. erythraeum, with main constituent (E)-2-decenal (52.22%), showed larvicidal activity. The main components of the essential oils of the fruits and herbal parts of Cymbocarpum wiedemannii were found to be aliphatic aldehydes and aliphatic acids.

A major cause of bacterial gastrointestinal infections is Helicobacter pylori. In fact, H. pylori has been designated as a class I carcinogen by World Health Organization and its eradication has been reported to be beneficial in preventing gastric disorders specially ulcer and cancer. Given the extensive treatment with antibiotics for decades, the failure rates due to antimicrobial resistance range from 20% to 40% and the eradication failure rate remains as high as 5-20%. Regarding the previous study which indicated that methanol extract of the plant showed antibacterial activity and anti-H. pylori activity of some flavonoids the present study was designed to evaluate anti-H. pylori property of the plant extracts and its flavonoids.

MATERIALS AND METHODS

Plant material

The flowering aerial parts of C. erythraeum were collected from the East Azerbaijan province (June 2010) with voucher No. 214 at the Herbarium of Institute of Medicinal Plants, Iranian Academic Centre for Education, Culture and Research, Karaj, Iran.

General

Silica gel (70-230 mesh, F254 pre-coated plates) (Merck, Germany), reverse phase silica gel 90 (RP-18C; Fluka, Switzerland) and Sephadex LH-20 (Fluka, Switzerland) were used for isolation of compounds. Semi-preparative high-performance liquid chromatography (HPLC (RP-18C; Knauer, Germany)) and medium-pressure liquid chromatography (MPLC (Silica gel, 230-400 mesh; Butchi, Switzerland)) were used for more purification. Nuclear magnetic resonance (NMR) experiments were performed on Bruker (Billerica, USA) DRX 500 instrument (500 MHz for proton NMR (1H-NMR), 125 MHz for carbon-13 (13C-NMR)) with tetramethylsilane as an internal standard. Ultraviolet (UV) spectra were measured on Optizen (Daejeon, Korea) model 2021 UV plus. All solvents were distilled before use.

Isolation procedure

Dried aerial parts of C. erythraeum (500 g) were extracted with hexane, methanol and water-methanol (1:1) using the solvent percolation method at room temperature. Extracts were concentrated to obtain 11, 80, 34 g of hexane, methanol and methanol-water (1:1) extracts, respectively. The methanol extract was further partitioned by petroleum ether, butanol and water. The butanol fraction (8 g) was subjected to Sephadex LH20 column to afford B1-B3 fractions and those suspected to contain flavonoids under UV light were loaded on another column for more purification. A sub-fractions (B1: 3980 mg, B2: 84 mg) were loaded on Sephadex LH20 column with methanol as eluent to obtain compounds 1 (8.4 mg) and 3 (12 mg). The other sub-fraction (B3: 106.6 mg) was injected to HPLC (RP-18C) eluted with different percentage of methanol-water (2:3, 1:1, and 3:2) to yield compound 2 (27 mg). In addition, the hexane extract was injected to MPLC (normal phase silica gel column) eluted with hexane-chloroform (4:1 and 1:1) to give 12 primary fractions (H1-H12). One fraction (H5: 239 mg) was subjected to Sephadex LH20 column with chloroform-ethyl acetate-methanol (1:1:1) as eluent to provide pure compound 4 (4.1 mg).

Anti-H. pylori assay

Clinical H. pylori (HP1, HP2, and HP3) strains were used to determine antimicrobial susceptibility following previously published protocols using the disk diffusion method. Serial dilutions of the test samples were made in dimethyl sulfoxide (DMSO). Suspensions of bacteria in normal saline were prepared with the turbidity of McFarland standard No. 2 (6×10⁸ cell/mL). Plates of non-selective blood agar were inoculated with 100 μL of each bacterial suspension and allowed to dry at room temperature. Ten μL of test samples was introduced into a sterile blank disks and deposited on the surface of the inoculated plates. Negative and positive control included blank disks impregnated with 10 μL of DMSO and amoxicillin (1 μg/mL), respectively. Plates were incubated at 37°C under microaerobic conditions and examined after 3-5 days. The mean inhibition zone diameters (IZD) ± standard deviation were recorded.

RESULTS AND DISCUSSION

The isolated compounds from aerial parts of C. erythraeum were identified as isoquercetin (1), rutin (2), along with β-sitosterol (3), and 2-decenol (4) based on the spectroscopic data (1H-NMR, 13C-NMR) and compared to the pertinent spectroscopic data in previously published literature (Figure 1). To the best of our knowledge, this is the first report on the isolation and elucidation of secondary metabolites of C. erythraeum.
Table 1. Inhibition zone diameters ± standard deviation of some active extracts of C. erythraeum against clinical isolates of H. pylori

| Samples | 1 Conc. | ½ Conc. | ¼ Conc. | 1/8 Conc. |
|---------|--------|--------|--------|--------|
|         | HP1    | HP2    | HP3    | HP1    |
| Hexane1 | 15±0.03| 17±0.04| 20±0.03| 14±0.05|
| Methanol2 | -     | 14±0.04| -      | 13±0.04|
| PE3     | -     | 15±0.02| -      | 12±0.03|

Conc.: Concentration, 1: 440 mg/mL, 2: 464 mg/mL, 3: 226 mg/mL. HP: Strains of H. pylori; PE: Petroleum ether. -: Not sensitive, IZD for amoxicillin (1 µg/mL): 30±0.04 mm; SD: Standard deviation.

O. H. p
11±0.03
10±0.03
16±0.04
9±0.01
12±0.05
10±0.02
12±0.05
10±0.02
12±0.05
11±0.03
10±0.02
12±0.05
10±0.02
12±0.05
11±0.03
10±0.02
12±0.05
10±0.02
12±0.05

Conflict of Interest: No conflict of interest was declared by the authors.

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