Chemical constituents of *Cymbocarpum erythraeum* (DC.) Boiss., and evaluation of its anti-*Helicobacter pylori* activity

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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-*Helicobacter 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 and Discussion:** The compounds were identified by ^{1}H-NMR and ^{13}C-NMR as isoquercetin (1), rutin (2), β-sitosterol (3) and 2-decenol (4). 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*

INTRODUCTION

Cymbocarpum is represented in Iran by three species that occur naturally in the wild, including *C. marginatum*, *C. erythraeum,* and *C. anethoides*.[1,2] 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.[1] Furthermore, the oil of *C. erythraeum*, with main constituent (*E*)-2-decenal (52.22%), showed larvicidal activity.[3] The main components of the essential oils of the fruits and herbal parts of *C. wiedemannii* were found to be aliphatic aldehydes and aliphatic acids.[4]

A major cause of bacterial gastrointestinal infections is *Helicobacter pylori*. In fact, *H. pylori* has been designated as a class I carcinogen by WHO and its eradication has been reported to be beneficial in preventing gastric disorders specially ulcer and cancer.[5] 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%.[6-8] Regarding the previous study which indicated that methanol extract of the plant showed antibacterial activity [9] and anti-*H. pylori* activity of some flavonoids [10] the present study was designed to evaluate anti-*H. pylori* property of the plant extracts and its flavonoids.

EXPERIMENTAL

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 (IMP), Iranian Academic Centre for Education, Culture and Research (ACECR), 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 HPLC (RP-18C; Knauer, Germany) and MPLC (Silica gel, 230-400 mesh; Butchi, Switzerland) were used for more purification. NMR experiments were performed on Bruker (Billerica, USA) DRX 500 instrument (500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR) with tetramethylsilane (TMS) as an internal standard. 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 LH_{20} column to afford B_A-B_E fractions and those suspected to contain flavonoids under UV light were loaded on another column for more purification. A sub-fractions (B_A: 3980 mg, B_B: 84 mg) were loaded on Sephadex LH_{20} column with methanol as eluent to obtain compounds **1** (8.4 mg) and **3** (12 mg). The other sub-fraction (B_C: 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 0:1) to give 12 primary fractions (H_A-H_L). One fraction (H_J: 239 mg) was subjected to Sephadex LH_{20} 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* (HP_1, HP_2, and HP_3) strains were used to determine antimicrobial susceptibility following previously published protocols using the disk diffusion method.[11,12] 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 (6×10^8 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**)[13,14], along with β-sitosterol (**3**)[15-17], and 2-decenol (**4**) based on the spectroscopic data (^{1}H-NMR, ^{13}C-NMR) and compared to the pertinent spectroscopic data in previously published literature (Fig. 1).[9]

Fig 1 here

To the best of our knowledge, this is the first report on the isolation and elucidation of secondary metabolites of *C. erythraeum* and its anti-*H. pylori* activity. The susceptibility of the tested bacteria were different related to the extracts and HP3 was the most susceptible strain. The inhibition zone diameters (IZD) of the effective test samples against *H. pylori* are summarized in (Table 1). The growth of all bacterial strains were suppressed by amoxicillin (1 µg/mL) with IZD of 30±0.04 mm, while DMSO showed no inhibitory activity toward the tested organisms. Methanol (464 mg/mL) and petroleum ether (226 mg/mL) extracts had inhibited one of the clinical isolates of *H. pylori* (HP3) with IZD of 14±0.04 and 15±0.02 mm, respectively, while compounds **1** (16 mg/mL), **2** (14 mg/mL) and the flavonoid fraction (138 mg/mL), along with other extracts were not effective against the isolated strains.

Table 1 here

In a previous study, rutin isolated from *Gardenia jasminoides* exhibited antibacterial activity toward *H. pylori*, however this present study showed the compound not to be active against the isolated strains.[18] Additionally, some flavonoids are known to be antibacterial agents.[19] For example, the flavonoid-rich extract of *Glycyrrhiza glabra* inhibited *H. pylori* growth by inhibition of protein synthesis, DNA gyrase and dihydrofolate reductase[10], while the flavonoid fraction of *C. erythraeum* was not active against examined strains, which may be presumably due to the difference in the flavonoid constituents of the plants and/or the susceptibility of bacteria. Whereas in this study, the hexane extract of the plant mainly inhibited the *H. pylori* strains attributed to the presence of non-polar compounds. It was reported that essential oil of *C. erythraeum*, which was rich in (*E*)-2-decenal (52.22%), was biologically active and exhibited larvicidal activity.[3] In the present study, the alcoholic compound 2-decenol (**4**), which was isolated from hexane fraction, is likely to be one of the active constituents of this fraction as well as other constituents in this fraction. The medium-chain-length alcohol decanol, from *Coriandrum sativum* oil, showed antibacterial activity especially against Gram-positive strains.[20,21] As a matter of fact, the relatively polar substances such as flavonoids and other polar fractions are not generally able to effectively prevent the bacterium growth, suggesting that the anti-*H. pylori* activity of this plant can be largely attributed to other non-polar secondary metabolites.

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