Fitoterapia 83 (2012) 1460–1465

Contents lists available at SciVerse ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

In vitro effects on intestinal bacterium of physalins from *Physalis alkekengi* var. Francheti

Xinli Li, Cuili Zhang, Dachang Wu, Li Tang, Xuejiao Cao, Yi Xin *

Department of Biotechnology, Dalian Medical University, Dalian, PR China

ARTICLE INFO

Article history:
Received 23 May 2012
Accepted in revised form 18 August 2012
Available online 25 August 2012

Keywords:
Physalis alkekengi var. Francheti
Physalin
Intestinal bacterium
Growth curve

ABSTRACT

Intestinal probiotic bacterium stimulative activity-guided fractionation of *Physalis alkekengi* var. Francheti calyces extract resulted in the isolation of four new physalins (**1**–**4**). Their structures were elucidated as 5α, 6β-dihydroxy-25, 27-dihydro-7-deoxyphysalin A (**1**), 5α, 6β-dihydroxyphysalin R (**2**), 3β-hydroxy-2-hydrophysalin A (**3**) and 5α-hydroxy-7-dehydro-25, 27-dihydro-7-deoxyneophysalin A (**4**) by UV, MS, 1D and 2D NMR spectroscopy. Growth curves of *Lactobacillus delbrueckii* and *Escherichia coli* for different total physalins extract (TPE) concentrations were tested *in vitro*. Middle concentrations (0.78 mg/mL–1.56 mg/mL) of TPE promoted the growth of *L. delbrueckii*, but all inhibited the growth of *E. coli*, in which the bacteriostatic activity increased while TPE concentration increases. Physalins showed stimulative effects on the growth of probiotic bacteria but inhibitory effects on the growth of pathogenic bacteria.

1. Introduction

Physalis alkekengi var. Francheti of the family of Solanaceae is a well-known edible and medicinal plant in the northeastern part of China. Its fruit calyx has been used in traditional Chinese medicine as a therapeutic agent for removing heat and toxic materials, and relieving sore-throat [1]. It has great potential and feasibly a stable form of *P. alkekengi* var. Francheti product could be developed to fulfill the health food market.

Studies on *P. alkekengi* var. Francheti have attracted more attention in recent years due to the potential biological functions. Active components such as physalins, polysaccharides and flavones have been investigated [2,3]. Physalin B has induced NOXA expression and then apoptosis in melanoma cells but not in non-tumor cells [4]. Physalin D, F and G have modulated lymphocyte functions in lymphoproliferative and cytokine production assays and in allogeneic transplantation model. [5] Physalin H has an immunosuppressive activity on T-cell activation and proliferation both *in vitro* and *in vivo* [6]. Physalin A and E have exhibited an anti-inflammatory action [7,8]. However, to the best of our knowledge, physalins with intestinal probiotic bacterium stimulative activity have not been studied.

In the present investigation on the EtOH extracted and macroporous resin refined extract of *P. alkekengi* var. Francheti, we describe the isolation and structural characterization by spectroscopic analyses of four new compounds: α, 6β-dihydroxy-25, 27-dihydro-7-deoxyphysalin A (**1**), 5α, 6β-dihydroxyphysalin R (**2**), 3β-hydroxy-2-hydrophysalin A (**3**) and 5α-hydroxy-7-dehydro-25, 27-dihydro-7-deoxyneophysalin A (**4**) (Fig. 1). Furthermore, the effects on intestinal bacterium of TPE were tested *in vitro*. Physalins showed stimulative effects on the growth of probiotic bacteria but inhibitory effects on the growth of pathogenic bacteria.

2. Experimental

2.1. General experimental procedures

Melting points were using an X-6 micromelting apparatus and are uncorrected. Optical rotations were measured using an AUTOPOL V digital polarimeter. UV spectra were obtained

* Corresponding author. Tel./fax: +86 411 86110295.
E-mail address: jimxinbio@gmail.com (Y. Xin).

0367-326X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fitote.2012.08.011

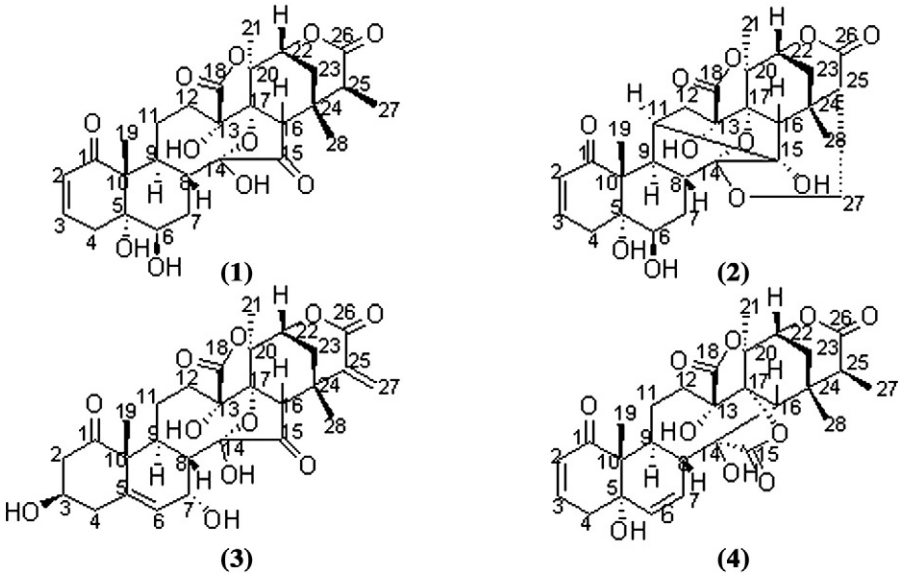

Fig. 1. The structures of **1–4** from *P. alkekengi* var. Francheti.

using a Jasco V-560 ultraviolet (UV)/vis spectrophotometer. ^{1}H NMR, ^{13}C NMR and 2D NMR spectra were recorded on a Bruker DRX-400 spectrometer. ESIMS data were obtained with a 4000 Q TRAP LC/MS/MS system by direct inlet using MeOH as solvent. Silica gel (200–300 mesh) used for column chromatography and Macroporous Resin AB-8 used for separation were supplied by the Qingdao Marine Chemical Factory China and Chemical Plant of Nankai University China, respectively. The column of HPLC was PREP-ODS 10×250 mm with a particle size of 5 μm (GL Sciences Inc., Tokyo, Japan). The OD values were measured by Thermo Labsystem MK3 Microplate Reader. Solvents were of industrial purity and distilled prior to use.

2.2. Bacteria strains

Strains of *Lactobacillus delbrueckii* ATCC 7830 and *Escherichia coli* ATCC 25922 were supplied by the Department of Biotechnology of Dalian Medical University.

2.3. Growth curves

L. delbrueckii ATCC 7830 and *E. coli* ATCC 25922 belong to the family of Lactobacillaceae and Enterobacteriaceae which were typical important intestinal microflora, respectively. They were cultured in MRS and LB medium respectively, and the medium was placed in the autoclave at 115 °C for 15 min.

The effect of total physalins on the growth of *L. delbrueckii* was assessed using 96-well microplates [9]. All wells added 100 μL MRS inoculated broth (2.25×10^{6} CFU/mL). The first well of each column was added of 100 μL of TPE to be tested and then 2-fold serial dilution was performed with the aid of a multichannel micropipette, resulting in final concentrations ranging from 6.25 mg/mL to 0.20 mg/mL. The microplates were incubated at 37 °C for 28 h and the OD values were performed at intervals of 2 h at 595 nm. Triplicates were made for each extract tested.

The effect of TPE on the growth of *E. coli* is similar to above, just all wells added 100 μL LB inoculated broth (1.25×10^{6} CFU/mL).

2.4. Plant material

The calyces of *P. alkekengi* var. Francheti were purchased from Dalian Traditional Chinese Medicine Market in October 2011 and identified by Yuling Yin, Professor of Department of Biotechnology, Dalian Medical University, China. A voucher specimen (no. LP201101) is deposited in the Department of Biotechnology, Dalian Medical University, China.

2.5. Extraction and isolation

The air dried calyces of *P. alkekengi* var. Francheti (3 kg) were extracted two times with 70% EtOH for 2 h at 60 °C. The solvent was evaporated to dryness, the dry residue (1.5 kg) was subjected to AB-8 macroporous adsorptive resin (12 kg), eluted with water (14 L), 50% EtOH (22 L), 95% EtOH (15 L) respectively to yield three fractions (*Frs.1–3*). *Fr.2* (165.0 g) was separated by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (10:1→1:1, v/v) to give nine fractions (*Frs.2.1–2.9*). *Fr.2.2* was recrystallized from MeOH to give **4** (30 mg). *Fr.2.3* was separated by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (8:1→1:1, v/v) to yield four fractions (*Frs.2.3.1–2.3.4*). *Fr.2.3.4* was further separated by HPLC with MeOH/H_2O (4:6) to give **1** (40 mg) and **2** (10 mg). *Fr.2.4* was separated by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (7:1→1:1, v/v) to yield give seven fractions (*Frs.2.4.1–2.4.7*). *Fr.2.4.2* was further separated by HPLC with MeOH/H_2O (1:1) to give **3** (12 mg).

Compound **1** (5α, 6β-dihydroxy-25, 27-dihydro-7-deoxyphysalin A) white amorphous powder, m.p. 270–272 °C. $[\alpha]^{20}_{D}$: −136 (*c* 0.2, MeOH). UV: 226 nm. ESIMS: *m/z* 564 $[M+NH_4]^{+}$. ^{1}H NMR and ^{13}C NMR data are given in Table 1.