สารต้านเอสโตรเจนจากสารสกัดขึ้นฉ่าย

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[2] สถานวิจัยความเป็นเลิศยาสมุนไพรและเทคโนโลยีชีวภาพทางเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ หาดใหญ่ สงขลา 90112

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บทคัดย่อ

นำสารประกอบ 6 ชนิด ได้แก่ junipediol A-8-*O*-β-D-glucoside (**1**), isofraxidin-β-D-glucoside (**2**), icariside D_2 (**3**), apiin (**4**), chrysoeriol 7-*O*-β-D-apiosylglucoside (**5**) และ 11,21-dioxo-3α,15β,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside (**6**) ซึ่งแยกได้จากสารสกัดหยาบด้วยเอทานอลของขึ้นฉ่าย มาทดสอบฤทธิ์ต้านเอสโตรเจน โดยใช้เซลล์มะเร็งเต้านมของมนุษย์ที่ตอบสนองต่อเอสโตรเจน (MCF-7) พบว่า junipediol A-8-*O*-β-D-glucoside และ 11,21-dioxo-3α,15β,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside มีฤทธิ์ดีที่สุดในการยับยั้งการเจริญเติบโตของเซลล์มะเร็งที่เกิดจากการกระตุ้นของเอสโตรเจน โดยสามารถยับยั้งการเจริญเติบโตได้ร้อยละ 83 และ 78 ตามลำดับ ที่ความเข้มข้นของสาร 100 ไมโครโมลาร์ ผลการศึกษานี้แสดงให้เห็นว่าผลของการกระตุ้นการหลั่งน้ำนมของขึ้นฉ่ายอาจมีความสัมพันธ์กับฤทธิ์ต้านเอสโตรเจนของสารทั้งสองในกรณีที่หญิงให้นมบุตรมีปริมาณน้ำนมไม่เพียงพอเนื่องจากมีการหลั่งเอสโตรเจนมากเกินไป

คำสำคัญ : ขึ้นฉ่าย, ฤทธิ์ต้านเอสโตรเจน, junipediol A-8-*O*-β-D-glucoside

Anti-estrogenic compounds from *Apium graveolens* L. extract

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Abstract

Six compounds, including junipediol A-8-*O*-β-D-glucoside (**1**), isofraxidin-β-D-glucoside (**2**), icariside D_2 (**3**), apiin (**4**), chrysoeriol 7-*O*-β-D-apiosylglucoside (**5**) and 11,21-dioxo-3α,15β,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside (**6**), isolated from the crude methanol extract of *Apium graveolens* L. were evaluated for their anti-estrogenic activity using the estrogen-responsive human breast cancer cell lines (MCF-7). Junipediol A-8-*O*-β-D-glucoside and 11,21-dioxo-3α,15β,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside showed the most potent inhibitory effect on estrogen-enhanced cell proliferation, with 83% and 78% inhibition, respectively, at 100 μM. These results indicated that the lactation-promoting property of *A. graveolens* might be related to the inhibitory effect of these compounds on excess estrogen in women who have insufficient milk for breastfeeding.

Keyword: *Apium graveolens*, anti-estrogenic, junipediol A-8-*O*-β-D-glucoside

บทนำ

Estrogen is a regulator for milk production in pregnant women. A high level of prolactin and a relatively low level of estradiol are observed during normal breastfeeding. A high level of estradiol can inhibit lactation by reducing milk secretion and by activating changes to the mammary secretory epithelial morphology. An inadequate milk production has often been observed in mothers who deliver preterm infants. Other factors such as diabetes, hypothyroidism, obesity, theca-lutein cysts, and polycystic ovarian syndrome may also lead to insufficient milk production.[1]

Apium graveolens L. (Apiaceae) has been used in Ayurvedic medicines as an anti-inflammatory, uricosuric, diuretic agent and for treatment of rheumatism. In addition, this plant has been used in a galactogogue preparation.[2] In this study we have focused on evaluation of the anti-estrogenic activity of six compounds isolated from the whole plant of *A. graveolens* using the estrogen-responsive MCF-7 cell lines. The results from this study may be a guide for their application as a lactation-promoting agent.

Materials and Methods

Chemicals and reagents

Fetal bovine serum (FBS) was from Gibco (Grand Island, NY). Eagle's MEM and RPMI media were from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). 17β-Estradiol and dextran-coated charcoal (DCC) were from Sigma Chemicals (St. Louis, MO).

Plant material

A. graveolens was obtained from a local fresh food market in Songkhla Province, Thailand in 2013. Voucher specimens (SKP 012 01 07 01) were deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The whole plants were dried at 50°C for 24 h in a hot air oven, and then reduced to coarse powders using a grinder.

Extraction and isolation of compounds from *A. graveolens*

Six compounds, including junipediol A-8-*O*-β-D-glucoside (**1**), isofraxidin-β-D-glucoside (**2**), icariside D_2 (**3**), apiin (**4**), chrysoeriol 7-*O*-β-D-apiosylglucoside (**5**) and 11,21-dioxo-3α,15β,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside (**6**) (Fig. 1) were isolated from the crude methanol extract of *A. graveolens* and identified using the methods previously described.[3]

Anti-estrogenic assay

The anti-estrogenic assay was conducted according to the procedure used for the cell proliferation assay.[1] MCF-7 cells were seeded at a density of 1×10^4 cells/well in a 96-well plate in 90 μL of 5% DCC-treated, FBS-supplemented RPMI phenol red-free medium. Then, 5 μL of estradiol (E_2) at a concentration of 20 nM was added into each well (final concentration of 100 pM), and the plates were incubated at 37°C in an atmosphere containing 5% CO_2 for 1 h. An adequate portion (5 μL) of each tested compound was added into each well to obtain a final concentration ranges of 0.1 - 100 μM, and incubated in a CO_2 incubator for 96 h. The antagonistic effects of the tested compounds were evaluated from a measure of the cell populations, and the percentage inhibition was determined for the required concentrations to inhibit the E_2 effect (the concentration suppressed the E_2 effect to the equivalent level of 100 pM). Values for the $iEqE_{50}$ of each sample were determined for the required concentrations to inhibit the E_2 effect (the concentration suppressing the E_2 effect to the equivalent level of 50 pM). When samples constantly suppressed E_2 activity to the level less than 10 or 50 pM through the concentrations tested, they were categorized as strong (S) or mild (M), respectively.

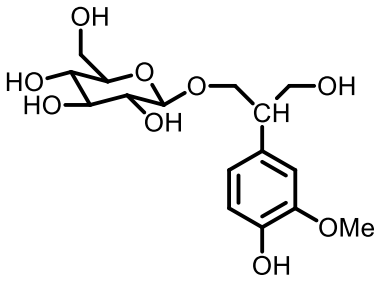
Results and discussion

Based on the results from the antiestrogenic assay using the estrogen-responsive MCF-7 cell line, Junipediol A-8-*O*-β-D-glucoside and 11,21-dioxo-3α,15β,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside showed significant antiestrogenic effects. They each exhibited mild inhibitory activity on the E_2-enhanced cell proliferation of the MCF-7 cell lines and low concentrations were found for their $iEqE_{50}$ values (Table 1). Our findings indicated that *A. graveolens* is an edible plant that may be used as a functional food to promote lactation by inhibiting the effect of estradiol in breastfeeding women.

The six compounds were also investigated for any estrogen-like stimulation of MCF-7 cell proliferation. Increasing concentrations of six compounds at 0.1, 1, 10, and 100 μM were tested using the MCF proliferation assay and compared with a positive control, estradiol (E_2), at concentrations that ranged from 1 to 100 pM. None of these compounds exhibited any significant stimulatory effects on MCF-7 cell proliferation (data not shown).

Acknowledgements

The authors wish to thank Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0120/2552) for financial support in this research. The authors would also like to acknowledge Dr. Brian Hodgson for assistance with the English.



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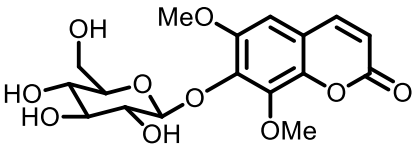

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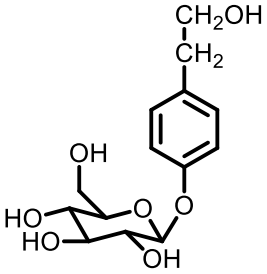

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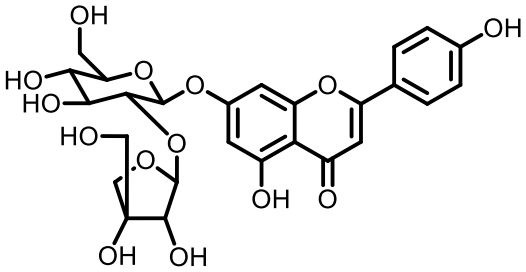

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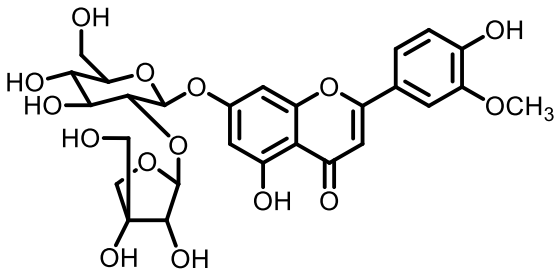

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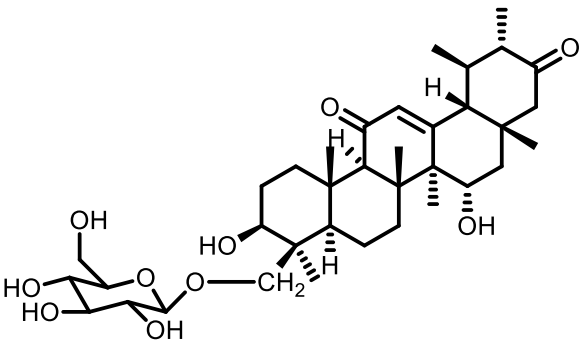

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Figure 1 Chemical structures of junipediol A-8-*O*-β-D-glucoside (**1**), isofraxidin-β-D-glucoside (**2**), icariside D_2 (**3**), apiin (**4**), chrysoeriol 7-*O*-β-D-apiosylglucoside (**5**) and 11,21-dioxo-3α,15β,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside (**6**)

Table 1 Inhibitory activity of six compounds isolated from *A. graveolens* against E_2-enhanced MCF-7 cell proliferation

Compounds	iEqE$_{50}$ (μM)[a]	IL[b]	Anti-estrogenic activity (%) at 100 μM
Junipediol A-8-*O*-β-D-glucoside	0.01	M[c]	82.9
Isofraxidin-β-D-glucoside	12.50	M[c]	54.7
Icariside D_2	>100		31.2
Apiin	84.58	M[c]	51.9
Chrysoeriol 7-*O*-β-D-apiosylglucoside	93.62		44.4
11,21-Dioxo-3β,15α,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside	0.03	M[c]	78.1

[a] iEqE$_{50}$ represented the concentrations of compounds that inhibited cell proliferation that was being enhanced by 100 pM of E_2 to the levels equivalent to those induced by 50 pM of E_2 treatment. These values were determined by linear regression analysis using four different concentrations.

[b] IL Inhibitory level of the compounds.

[c] Mild inhibition (M) = More than 50% inhibition through the concentrations tested.

References

1. Luecha, P., Umehara, K., Miyase, T., Noguchi, H. (2009). Antiestrogenic constituents of the Thai medicinal plants *Capparis flavicans* and *Vitex glabrata* **J. Nat. Prod.** 72 (11), 1954-1959.
2. Asif, H. M., Akram, M., Usmanghani, K., Akhtar, N., Shah, P. A., Uzair, M., Ramzan, M., Shah, S. M. A., Rehman, R. (2011). Monograph of *Apium graveolens* Linn. **J. Med. Plant Res.** 5 (8), 1494-1496.
3. Simaratanamongkol, A., Umehara, K., Noguchi, H., Panichayupakaranant, P. (2014). Identification of a new angiotensin-converting enzyme (ACE) inhibitor from Thai edible plants. **Food Chem**. 165, 92-97.