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The Estrogenic-Like Activity of Four Chinese Medicinal Plants in Vitex

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Abstract: In most developing countries, 70-80% of the populations still resort to traditional medicine for their primary health care. This medicine utilizes medicinal plants which are traditionally taken as concoction and infusion. Ethanolic extracts of selected four Chinese medicinal plants in Vitex were tested for proliferative activity in ER_a-positive MCF-7 human cell line using MTT assay. *Vitex negundo* showed the most estrogenic-like potent activity, which could be useful as a Hormone Replacement Therapy (HRP). In further investigation, the compounds with activities in *Vitex negundo* will be elucidated.

Key words: Vitex, estrogenic-like activity, proliferative activity, HPP, *Vitex negundo*

INTRODUCTION

Phytoestrogens are plant-derived compounds with estrogenic or antiestrogenic properties (Umland *et al*., 2000). The scientific interest in phytoestrogens is enhanced by the hope for a potential medical use like in Hormone Replacement Therapy (HRT) with litter side effects. Phytoestrogens are then hoped to be beneficial to relieve a variety of symptoms of menopause and help prevent bone resorption.

As part of our continuing search for anti-PMS and estrogenic activity from medicinal plants, *Vitex Agnus-Castus* is an ancient medicinal plant for a variety of gynecologic conditions and its fruit (VAC) extract has been used traditionally in the treatment of conditions affecting females including menstrual disorders, corpus luteum insufficiency, menopause and hormonal imbalance in Europe (Daniele *et al*., 2005). While in China, *Vitex rotundifolia* L., *Vitex trifolia* L., *Vitex negundo* L. and *Vitex negundo* var. *cannabifolia* Hands-Mazz., which belongs to the same genus as *V. agnus-castus* and are also the member of the *Vitex* (Verbenaceae), have the similarity in their morphological and histological characters with *V. agnus-castus* (Wu *et al*., 1994). The main constituents of the fruits of these four medicinal plants have been determined to be flavonoids, terpenoids, glucosides, which are similar to the components of *V. agnus castus* (Singh *et al*., 2003; Ono *et al*., 2001; Masateru *et al*., 2001; Leitao *et al*., 1999). The fruits, leaves and stems of these *Vitex* plants are frequently used in Traditional Chinese Medicine (TCM)as preventatives for the common cold, coughs, asthma, chronic bronchitis and gastrointestinal infections (Li *et al*., 2005). While, the goal of this study was to investigate if the four medicinal plants in close relationship with *V. agnus-castus* in traditional taxonomy have potential estrogenic activity, like *V. agnus-castus,* using MTT assay in MCF-7 human breast adenocarcinoma cell.

MATERIALS AND MEDTHODS

Plant material and extraction: Plants, listed in Table 1, were collected from different sites in China and authenticated by Prof. Hanchen Zheng, Second Military Medical University. The voucher specimens of these plants were deposited at the Herbarium of Department of Pharmacognosy, Second Military Medical University, Shanghai, PR China. The powdered fruits material (2900g) were successively infiltrated with 60% EtOH at room temperature for 2 weeks and evaporated under vacuum to obtain the EtOH extract. Yields of ethanolic extract from tested material were also listed in Table 1.

Cell culture: Estrogen receptor-positive human breast adenocarcinoma MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing antibiotics (100 IU mL^{-1} penicillin and 100 μg mL^{-1} streptomycin), supplemented with 10% fetal bovine serum. Cells were grown at 37 in a humidified atmosphere of 95% air/5% CO2 and the medium was renewed 2-3 times per week.

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Table 1: Plants collected from different locations in China and their weight/weight yields in terms of crude medicinal materials

Tested materials	Locality	Location (latitude, longitude)	Yield (%)EtOH
Vitex rotundifolia (Fruits)	Xinjian, Jiangxi Province	28°25.41'N, 115°48.61'E	12.1
Vitex trifolia (Fruits)	Shenzhen, Guangdong Province	23°44.60'N, 117°21.25'E	11.6
Vitex negundo (Fruits)	Luzhou, Siochuan Province	31°61.60'N, 105°21.15'E	9.5
Vitex negundo var. *cannabifolia* (Fruits)	Jiande, Zhejiang Province	29°17.65'N, 119°10.12'E	10.9

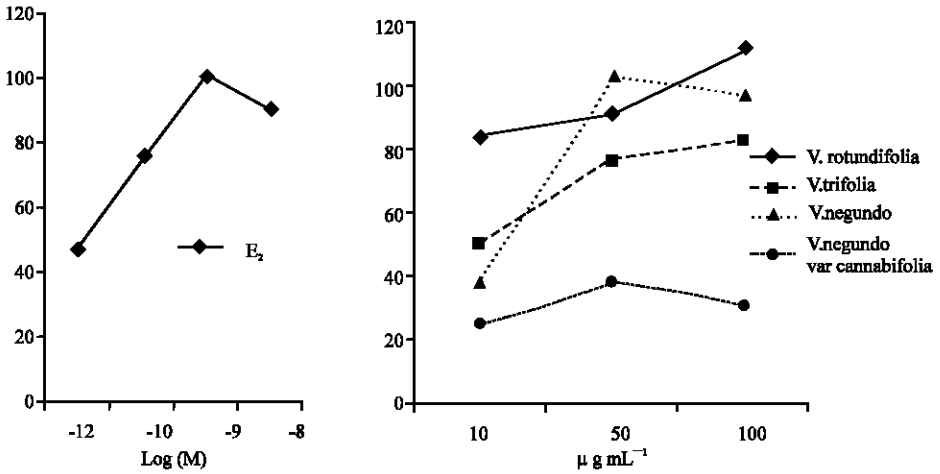

Fig. 1: Effects of the extract from four medicinal plants on the proliferation of ER-positive human breast cancer cells. The cells were incubated in phenol red-free DMEM supplemented with hormone-free human serum with VRE and test compounds for 7 days. After incubation for 7 days, the MTT assay was performed to measure cell proliferation. The proliferative effect relative to estradiol (1 nM, 100%) is expressed as Relative Proliferative Effect (RPE)

Charcoal-dextran stripped human serum preparation: In order to minimize the estrogenic activity of serum, steroid hormones were stripped from pooled human serum by treatment with charcoal and dextran (purchased from Gibco BRL). The charcoal-dextran stripped human serum was filtered and stored at -20□ until used.

Proliferation assay of MCF-7 cells: Confluent MCF-7 cells were washed twice with D-Hanks solution before the addition of 0.25% trypsin-EDTA. The flask was left for 2-3 min at room temperature(close to 20 □), after which the cells were detached, resuspended in full medium, counted and seeded into 96-well plates at a density of 1×10^4 cells/well in normal growth medium. After 48 h, the cells were completely attached to the well bottom. The cells were then washed with D-Hanks and the estrogen-free medium (phenol redfree DMEM with 5% charcoal-dextran stripped human serum) was added and cultured for 24 h, the different concentrations of test materials were also added to this medium. In the antagonistic test of the cell proliferation assay, the pure estrogen receptor antagonist-0.1μM ICI 182,780 [7α-[9(4, 4, 5, 5, 5-pentafluoropentyl) sulfinyl]nonyl) -estra-1, 3, 5 (10)-triene-3, 17β-diol)] was added with the test compounds. Cell proliferation was assessed after 7 days, during which the medium was changed every 3 days. In the assessment method, cells were incubated with 100 μL of 5 mg mL^{-1} 3-(4, 5-dimethylthiazol -2-yl) -2, 5 -diphenyl-tetrazolium bromile (MTT) solution for 4 h. The medium was the discarded and replaced with 600 μL DMSO. The absorbance was measured at 540 nm in an ELx800 universal micro plate reader (Bio-TEK, USA) and cell proliferation was expressed as absorbance values. The results are expressed as proliferation compared with that induced by treatment with 1 nM estradiol.

RESULTS AND DISCUSSION

The estrogenic effect of the extracted was examined by using the E-SCREEN assay in MCF-7 cells. The proliferative effect of the extracts relative to that of estradiol (1 nM, 100%) is expressed as Relative Proliferative Effect (RPE) (Fig. 1). Figure 1 show clearly the extracts from *V. rotundifolia* and *V. negundo* were able to significantly stimulate MCF-7 cell proliferation at concentrations of 50 μg mL^{-1} to100 μg mL^{-1} ($p<0.01$) (Fig. 1). Two best proliferative effects of the extract from *V. rotundifolia* and *V. negundo* were achieved

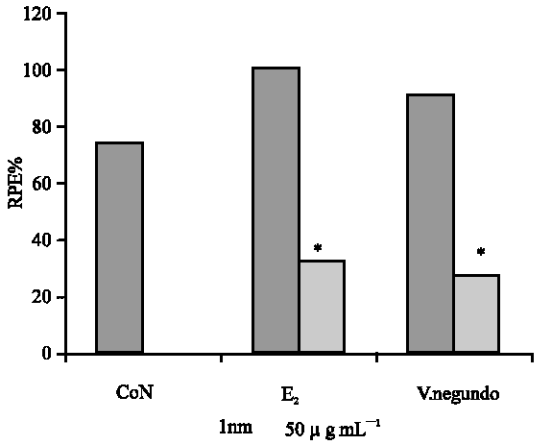

Fig. 2: Effect of cotreatment with pure antiestrogen ICI 182,780 on cell proliferation induced by the extract of *V. negundo* in MCF-7 cells. The cells were incubated in phenol red-free DMEM supplemented with hormone-free human serum without or with 100 nM ICI 182,780 for 7 days. After incubation for 7 days, the MTT assay was performed to measure cell proliferation. The proliferative effect relative to estradiol (1 nM, 100%) is expressed as Relative Proliferative Effect (RPE). Results are expressed as means±SD of five separate experiments for each data point. Significance was set at * $p<0.01$ vs. the same dose of extract without treatment of ICI 182,780

at 100 μg mL^{-1} (RPE%=106.2±12.3%) and 50 μg mL^{-1} (RPE%=96.25±15.1%), respectively, which were almost equivalent to the effect displayed by 1 nM estradiol. But combining the concentration and the proliferative effect, *V. negundo* displayed the most potent estrogenic-like activity. And 50 mg L^{-1} concentration of the extract from *V. negundo* could be reversed by co-administration of a pure anti-estrogen ICI 182,780 (Fig. 2). The RPE of the extract from *V. negundo* after treatment with ICI 182,780 decreased to 27.7±7.0%.

Phytoestrogens are polyphenolic non-steroidal plant derived compounds with estrogen-like biological activity, which have been associated with a variety of changes in the reproductive system and certain hormone-dependent diseases, such as prostate cancer, colon cancer, breast cancer and PMS (Laura *et al*., 2001; Cos *et al*., 2003). The result showed that, in the four medicinal plants, the ethanolic extract of *V. negundo* could significantly stimulate the growth of MCF-7 cells and the proliferation stimulatory effect could be reversed by co-administration of a pure anti-estrogen ICI 182,780. Thus, it can be concluded that extract of *V. negundo* possess potential estrogen-like activity in MCF-7 cells and as phytoestrogens, it may have a role to play in the treatment of PMS and menopausal disorders by regulating the levels of the estrogen and progesterone. Further test of the estrogenic activity of the compounds in *V. negundo* is currently being conducted in our laboratory. This study also suggested that it may be a shortcut to find the similar activities in close relationship medicinal plant in traditional taxonomy.

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