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AROMATASE INHIBITORY AND CYTOTOXIC ACTIVITIES OF CHEMICAL CONSTITUENTS FROM THE VIETNAMESE MEDICINAL PLANT BAN-CHI-LIEN (*SCUTELLARIA BARBATA* D. DON)

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ABSTRACT

Aromatase inhibitory and cytotoxic activities were determined for apigenin, luteolin and the new diterpene named scutebarbalactone VN, which were obtained by bioassay-guided fractionation and isolation from the methanol extract of the Vietnamese medicinal plant Ban-chi-lien (*Scutellaria barbata* D. Don). In the aromatase inhibition assay, an IC_{50} value of 3.36 μM was found for scutebarbalactone VN, while IC_{50} values of 7.2 μM and 7.95 μM were found for the positive controls aminoglutethimide and β-estradiol, respectively. In the cytotoxicity assays using a panel of human cancer cell lines, scutebarbalactone VN showed promising anticancer activity with IC_{50} ranging from 2.15 to 8.3 μM compared with those of the positive control ellipticine ranging from 1.0 to 2.1 μM. Apigenin and luteolin were found to be inactive in both assays.

Key words: *Scutellaria barbata*, aromatase inhibitory activity, cytotoxic activity, scutebarbalactone VN, apigenin, luteolin, aminoglutethimide, β-estradiol, ellipticine.

1. INTRODUCTION

There is an increasing need in Vietnam for anti-cancer and cancer preventive medicines, but only about 10 % of the Vietnamese cancer patients have access to modern therapy and medicaments [1]. The major part still relies on traditional herbal medicines for their cure. Therefore it is our desire to investigate those plants scientifically, so that they could be used more effectively.

Our biological screening of Vietnamese medicinal plants with a traditional use to treat tumor using the cytotoxicity assay revealed that the plant Ban-chi-lien (*Scutellaria barbata* D. Don, also known under the synonym *Scutellaria rivularis* Wall., family Lamiaceae) possesses

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remarkable anti-cancer properties. Bioassay-guided fractionation of the methanol extract of the whole plant then resulted in the isolation of a new neoclerodane diterpenelactone, named as scutebarbalactone VN, together with the flavones apigenin and luteolin. Their isolation and structure elucidation were described in a separate report [2]. In this article we report about the aromatase inhibitory and cytotoxic activities of these chemical constituents, as well as about the bioassay-guided fractionation and isolation, which led to them.

2. EXPERIMENTAL SECTION

2.1 Plant material

The whole plants of *Scutellaria barbata* were collected in Hoa Binh province in November 2003. A voucher specimen (accession code BCL1) is deposited at the Department of Animal Cell Technology, Institute of Biotechnology, VAST, Hanoi, Vietnam. After the collection, the whole plants were air-dried, stored in an air-conditioned storage room, and powdered mechanically just before extraction.

2.2 Extraction and Isolation:

The aqueous extract was made according to traditional preparation method by gentle boiling of 100 g of the air-dried and powdered plant sample with water in about 10 hours. Water was then removed from the extract by evaporation *in vacuo* to yield a water extract residue. The methanol extract was prepared by extraction with methanol at room temperature until exhaustion. The combined methanol extracts were concentrated *in vacuo* at 40 ^{o}C to yield the methanol extract residue.

For the bioassay-guided fractionation, the methanol extract residue was partitioned successively between n-hexane, dichloromethane and water. The dichloromethane extract was chromatographed on a Sephadex LH-20 column and eluted with methanol to yield 4 combined fractions **R4** - **R7**.

The chemical constituents apigenin (**1**), luteolin (**2**) and scutebarbalactone VN (**3**) were isolated from the active fractions.

2.3 Assay for Inhibition of Aromatase activity:

Aromatase inhibitory activity was determined by measuring the fluorescent intensity of the product, which is formed from the dibenzylfluorescein (DBF) dealkylation under the catalysis of aromatase [3]. The assay was conducted in 96-well microtiter plates (Cat # 3915, Corning Costar). The substrate DBF and aromatase enzyme (baculovirus / insect cell-expressed) were obtained from Gentest Corporation. A cofactor/serial dilution (C/SD) buffer was prepared in 50 mM potassium phosphate, pH 7.4. This buffer contains 2.6 mM $NADP^{+}$, 6.6 mM glucose-6-phosphate, 6.6 mM $MgCl_2$, 4 unit/ml of glucose-6-phosphate dehydrogenase, and 1 mg/ml albumin. 100 µl of test substance or extract and C/SD buffer in the ratio of 1:9 were added. The plate was then pre-incubated at 37 ^{o}C for 10 minutes, and the reaction was initiated by addition of 100 µl of a pre-warmed enzyme/substrate (E/S) mixture. The E/S mixture contained potassium phosphate buffer (pH 7.4), DBF (0.2 µM final), enzyme (2 pmol/ml final) and 4 mg/ml albumin. The plate was continued to be incubated at 37 ^{o}C for 30 minutes. Reactions were terminated by addition of 75 µl 2 N NaOH. To develop adequate signal to background ratio, the plate (with lid) was then incubated at 37^{o}C for 2 hours. Fluorescence was measured at 485 nm excitation and 538 nm emission wavelength with a fluorescence plate reader. Data were exported and analyzed using an Excel spreadsheet and the IC_{50} values were determined by the TableCurve software.

Samples were tested in triplicate and the mean values were used to prepare dose – response curves. Aminoglutethimide, Naringenin, β-estradiol (Sigma, St. Louis, MO) were used as positive controls.

2.4 Cytotoxicity assay

Cytotoxicity assays were performed according to a method developed by Monks, which is being used at the National Institute of Health (USA) as a standard method for the evaluation of the cytotoxic potential of compounds or extracts using a panel of human cancer cell lines [4]. Cell lines were grown in 96-well microtiter plates with each well containing 190 µl medium. After 24 hrs, 10 µl of the test samples dissolved in 10% DMSO were added to the wells. The cells were then cultured for additional 48 hrs, fixed with trichloroacetic acid, and stained with sulforhodamine B, followed by the determination of the optical densities at 515 nm using a Microplate Reader (BioRad). The cytotoxicities were calculated and expressed as IC_{50} values. Extracts and fractions having an IC_{50} < 20 µg/ml and pure compounds having an IC_{50} < 10 µM were considered as cytotoxicity active. The cancer cell lines KB (human epidermoid carcinoma), LNCaP (hormone dependent prostate cancer), LU (human lung cancer), MCF7 (human breast cancer) were used for the assays. Ellipticine was used as a positive control.

3. RESULTS AND DISCUSSION

3.1 Bioassay-guided fractionation and isolation of chemical constituents

Primary biological screenings of the extracts (see Tables 1 and 2) showed that *S. barbata* was active in both aromatase inhibitory and cytotoxic assays, and that the activities were retained in the methanol extract, while the water extract showed no activity.

Table 1: *Aromatase Inhibitory Activity of the Primary Extracts.*

Sample	IC_{50} (µg/ml)
Methanol Extract Residue	11.63
Water Extract Residue	>20
Naringenin (positive control)	0.36

Table 2: *Cytotoxicity of the Primary Extracts, IC50 (µg/ml).*

Sample	KB	LU	LNCaP	MCF7
Methanol Extract Residue	17.2	12.7	13.82	10.15
Water Extract Residue	>20	>20	>20	>20
Ellipticine (positive control)	0.98	0.58	0.56	0.45

The fractionation of the active methanol extract was shown in Figure 1 and performed as described in the experimental section.

In order to guide chemical isolation, cytotoxicity assays of the fractions were performed. The results were shown on Table 3.

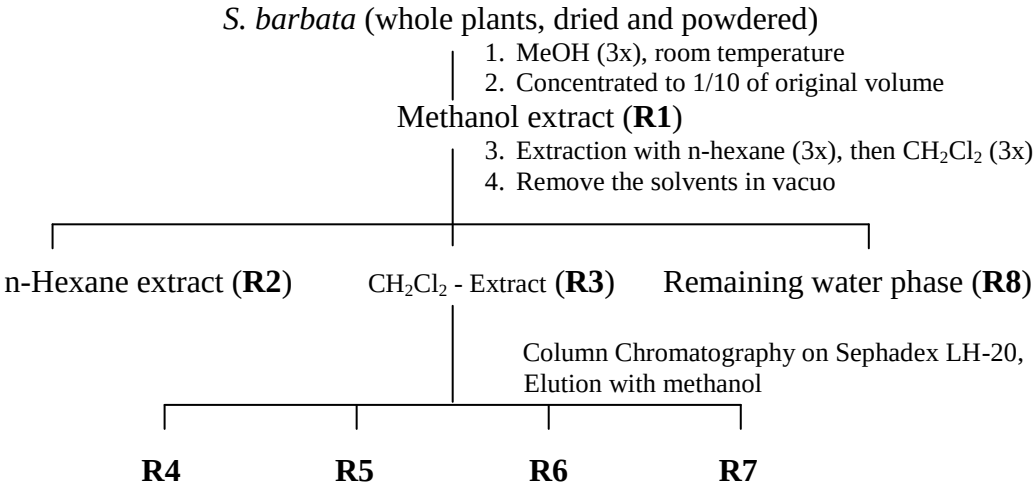
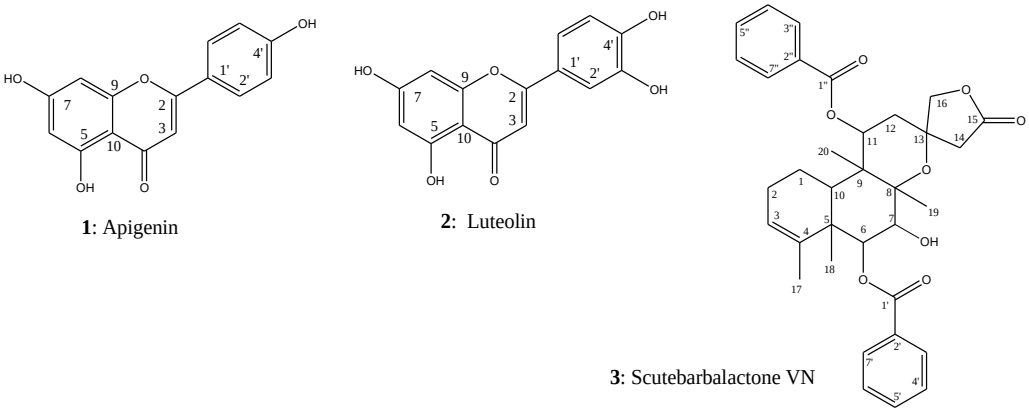


Fig. 1: *Bioassay-guided fractionation of S. barbata.*

Table 3: *Cytotoxicity of the Fractions, IC_{50} (μg/ml).*

Fraction	KB	LU	MCF7	LNCaP	Activity
R1 (methanol extract)	19.8	16.67	17.99	19.89	Active
R2	19.27	>20	5.98	>20	Active
R3	15.24	18.9	3.24	14.74	Active
R4	>20	>20	>20	>20	Not active
R5	14.68	18.41	2.08	13.2	Active
R6	>20	17.06	2.48	17.95	Active
R7	>20	>20	>20	>20	Not active
R8	>20	>20	>20	>20	Not active
Ellipticine (positive control)	0.62	0.52	0.3	0.54	Very active



1: Apigenin

2: Luteolin

3: Scutebarbalactone VN

Fig.2*: Isolated Chemical Constituents.*

From the active fractions, the chemical constituents apigenin (**1**), luteolin (**2**) and scutebarbalactone VN (**3**) were isolated (see Figure 2).

3.2 Aromatase Inhibitory and Cytotoxic Activities of the Chemical Constituents

The aromatase inhibitory and cytotoxic activities of the isolated compounds **1** - **3** were determined and presented in Table 4 and 5. In the aromatase inhibition assay, an IC_{50} value of 3.36 μM was found for scutebarbalactone VN (**3**), while IC_{50} values of 7.2 μM and 7.95 μM were found for the positive controls aminoglutethimide and β-estradiol, respectively. In the cytotoxicity assays scutebarbalactone VN showed promising anticancer activity with IC_{50} ranging from 2.15 to 8.3 μM compared with those of the positive control ellipticine ranging from 1.0 to 2.1 μM. Apigenin (**1**) and luteolin (**2**) were found to be inactive in both assays.

Table 4:*Aromatase Inhibitory Activity of the Chemical Constituents.*

Compound	IC_{50} (μM)	Activity
Apigenin (**1**)	>20	Not active
Luteolin (**2**)	>20	Not active
Scutebarbalactone VN (**3**)	3.36	Active
Aminoglutethimide (positive control)	7.2	Active
β-estradiol (positive control)	7.95	Active

Table 5:*Cytotoxicity of the Chemical Constituents.*

Compound	KB	LU	MCF7	LNCaP	Activity
Apigenin (**1**)	>10	>10	>10	>10	Not active
Luteolin (**2**)	>10	>10	>10	>10	Not active
Scutebarbalactone VN (**3**)	8.3	7.2	2.15	7.5	Active
Ellipticine (positive control)	2.1	1.74	1.0	1.8	Active

4. CONCLUSIONS AND DISCUSSION

In conclusion, bioassay-guided fractionation of a methanol extract from the whole plant of *Scutellaria barbata D. Don* led to the isolation of one new diterpene compound scutebarbalactone VN, and two known flavones apigenin and luteolin. The new compound scutebarbalactone VN was shown to possess strong aromatase inhibitory activity with an IC_{50} value of 3.36 μM and an evidently selective cytotoxic activity against the breast cancer cell line MCF7 (IC_{50} = 2.15 μM).

Despite its popularity in traditional medicine, the Vietnamese *S. barbata* has not been scientifically studied before, while plants of other origins, especially the Chinese plant, have been the subject of many scientific investigations. Recent findings have been published, showing its anticancer properties in various human cancers [5], especially in breast [6], ovarian

[7], lung [8], colon [9] and cervix cancers [10]. Characteristic compounds were found to be flavonoids and neoclerodane diterpenelactones. The observed activities were mostly assigned to the flavonoid constituents like apigenin, luteolin and others [11], while the biological activities of the neoclerodane diterpenelactones have not yet been studied.

The results of this study on the Vietnamese plant *S. barbata* demonstrated that: a) The anti-cancer activities of the plant and its chemical constituents are remarkable and therefore the plant deserves further investigations. b) Scutebarbalactone VN is worth to serve as a lead for future drug development against estrogen-dependent cancers; c) The Vietnamese plant contains similar (apigenin and luteolin) but also different (scutebarbalactone VN) chemical constituents than the Chinese plant; d) Any conclusions obtained with the Chinese plant should be applied with cautions when transferring to the Vietnamese plant.

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