

CYTOTOXIC EVALUATION OF 17 β H-NERIIFOLIN FROM *Cerbera odollam* AGAINST BREAST AND OVARIAN CANCER CELL LINES

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OBJECTIVE

1. To extract and fractionate five Malaysian plant species for anticancer evaluation.
2. To evaluate the anticancer activities of the plant species against a panel of breast and ovarian cancer cell lines.
3. To obtain the chemical profiles of the bioactive compound
4. To isolate and determine the bioactive compound as anticancer lead compound

MATERIALS AND METHOD

Plant materials

The fresh leaves of *Cerbera odollam* (Co), *Andrographis paniculata* (Ap), *Eurycoma longifolia* (El), *Macaranga pruinosa* (Mp) and *Mallotus macrostachyus* (Mm) were collected from Pantai Hiburan Rompin, Pahang. Voucher specimens were deposited at the herbarium of Forest Research Institute Malaysia (FRIM). The dried plant leaves were subjected to bioassay guided fractionation and isolation process.

Sample Preparation

The fresh leaves were cleaned and dried in an oven at 40-50°C. Dried samples were ground into powder to obtain powdered samples (40-60 mesh). 250 g of each sample were then extracted with 2000 ml of methanol (ratio: 1:8) using soxhlet extraction for 18 hrs, in 60°C. The liquid extract was concentrated using rotary vacuum evaporator to obtain methanolic crude extract. The crude extract obtained was then separated by liquid-liquid partitioning using ethyl acetate, n-butanol and water to obtain three fractions and subjected to cytotoxicity analysis.

Cell Culture

The cells were purchased from the American Type Culture Collection (ATCC, USA) and maintained in Dulbecco's Modified Essential Medium (DMEM, PAA lab, UK), supplemented with antibiotics (Flowlab, Australia) and grown at 37°C in a regulated 5% CO₂ incubator, until it reached its confluent stage. The routine maintenance of the cells was performed in accordance with the protocol of ATCC.

Cytotoxicity Assay

Samples were dissolved in ethanol (0.5% v/v) individually and diluted to produce five different concentrations of samples. The diluted samples were subjected to cytotoxicity assay in order to determine the IC₅₀ value of the samples against the breast cancer and ovarian cancer cell lines. An IC₅₀ value is the concentration of the sample to inhibit the population of the cell by 50%. Cultured cells 1x10⁵ per well were treated with the diluted samples and incubated for 72 hours in 5% CO₂ before the cells were assayed using the Sulforhodamine B (SRB) assay (Skehan *et al.* 1990). The plates were then analyzed using Magellan V.4 Elisa reader and software at 492 nm to determine the optical density that indicated the number of living cells.

Isolation and Structure Determination

The ethyl acetate fraction of *C.odollam* was fractionated by vacuum liquid chromatography (VLC) and 16 fractions obtained. The combined active fractions were then purified by column chromatography (CC) afforded active compound, 17βH-neriifolin. Spectroscopic and spectrometric analysis such as 1D/2D NMR and ESI-MS/MS were conducted to confirm the structure of the anticancer compound.

RESULTS

Bioprospecting of five Malaysian plant species; *Cerbera odollam*, *Andrographis paniculata*, *Eurycoma longifolia*, *Macaranga pruinosa* and *Mallotus macrostachyus* using bioassay-guided fractionation were evaluated against breast (MCF7 & T47D) and ovarian (SKOV-3 & CaOV3) cancer cell lines. Methanolic extract of *C.odollam* was determined as the most active extract and the bioactive compound was identified as 17βH-neriifolin. The IC₅₀ values of 17βH-neriifolin against MCF7, T47D, SKOV-3 & CaOV3 and normal kidney cell line (Vero) are 0.017, 0.021, 0.028, 0.032 and 0.024 μM, respectively. Whereas, tamoxifen; currently used as chemotherapy drug when treated with these cell lines exhibited IC₅₀ values of 13.7, 14.3, 13.2, 13.5, and 14.5 μM, respectively, which means that 17βH-neriifolin is 400 to 800 times more active than tamoxifen. This finding has been proven for its novelty and invention disclosure was filed. Further studies of 17βH-neriifolin, with the assessment of its toxicity and structure-activity relationships, might expand the possibilities of using 17βH-neriifolin as a novel therapeutic option for cancer therapy. As a conclusion, 17βH-neriifolin isolated from the leaves of *Cerbera odollam* exhibited its potential for biopharmaceutical development.