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Short communication

1′S-1′-Acetoxyeugenol acetate: A new chemotherapeutic natural compound against MCF-7 human breast cancer cells

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ABSTRACT

Medicinal plants containing active natural compounds have been used as an alternative treatment for cancer patients in many parts of the world especially in Asia (Itharat et al. 2004). In this report, we describe the cytotoxic and apoptotic properties of 1′S-1′-acetoxyeugenol acetate (AEA), an analogue of 1′S-1′-acetoxychavicol acetate (ACA), isolated from the Malaysian ethno-medicinal plant *Alpinia conchigera* Griff (Zingiberaceae) on human breast cancer cells. Data from MTT cell viability assays indicated that AEA induced both time- and dose-dependant cytotoxicity with an IC_{50} value of 14.0 μM within 36 h of treatment on MCF-7 cells, but not in HMEC normal control cells. Both annexin V-FITC/PI flow cytometric analysis and DNA fragmentation assays confirmed that AEA induced cell death via apoptosis. AEA was also found to induce cell cycle arrest in MCF-7 cells at the G_0/G_1 phase with no adverse cell cycle arrest effects on HMEC normal control cells. It was concluded that AEA isolated from the Malaysian tropical ginger represents a potential chemotherapeutic agent against human breast cancer cells with higher cytotoxicity potency than its analogue, ACA.

Introduction

Alpinia conchigera, also known locally as '*lengkuas ranting*', '*lengkuas kecil*', '*lengkuas padang*', '*lengkuas getting*' or '*chengkenam*' (Janssen and Scheffer 1985) is a herbaceous perennial, 2–5 feet tall, found in eastern Bengal and southwards to Peninsular Malaysia and Sumatera (Burkill 1966). It is used as a condiment in the northern states of Peninsular Malaysia and occasionally in traditional medicine in the east coast to treat fungal infections. In Thailand, the rhizomes are used in traditional Thai medicine to relieve the gastro-intestinal disorders and in the preparation of Thai food dishes (Matsuda et al. 2005).

Traditional medicine from various plant types containing active natural compounds such as chalcones (Hsu et al. 2006), xanthoangelol (Tabata et al. 2005) and licochalcone-A (Fu et al. 2004) have all been reported as potential drugs for the treatment of cancer. In recent years, the pro-apoptotic effects of 1′S-1′-acetoxychavicol acetate (ACA) from the Thai ginger isolate, *Languas galanga* and *Alpinia galanga*, have been documented in human breast carcinoma cells (Campbell et al. 2007), human T cell lymphoma (Ichikawa et al. 2005) and in the inhibition of tumor-promoter-induced Epstein–Barr virus (Kondo et al. 1993). Even though previous studies have shown that ACA isolates from ginger exhibited anti-tumor properties against a wide variety of cancers, there have been no reports thus far on the cytotoxic and apoptotic effects of the closely related 1′S-1′-aceotxyeugenol acetate (AEA) (Fig. 1) on human breast cancer cells.

This study describes the purification, characterization and biological activity of six natural ACA analogs isolated from the rhizomes of the Malaysian wild-ginger, *Alpinia conchigera* (Zingiberaceae). We report herein the phytochemical data as well as our preliminary cytotoxic and apoptotic effects of AEA (Fig. 2) on MCF-7 human breast cancer cells.

Materials and methods

Plant material

Rhizomes of *Alpinia conchigera* Griff. were collected from Jeli province of Kelantan, East-coast of Peninsular Malaysia. The sample was identified by Prof. Dr. Halijah Ibrahim from the Institute of Biological Science, Faculty of Science, University Malaya. A voucher

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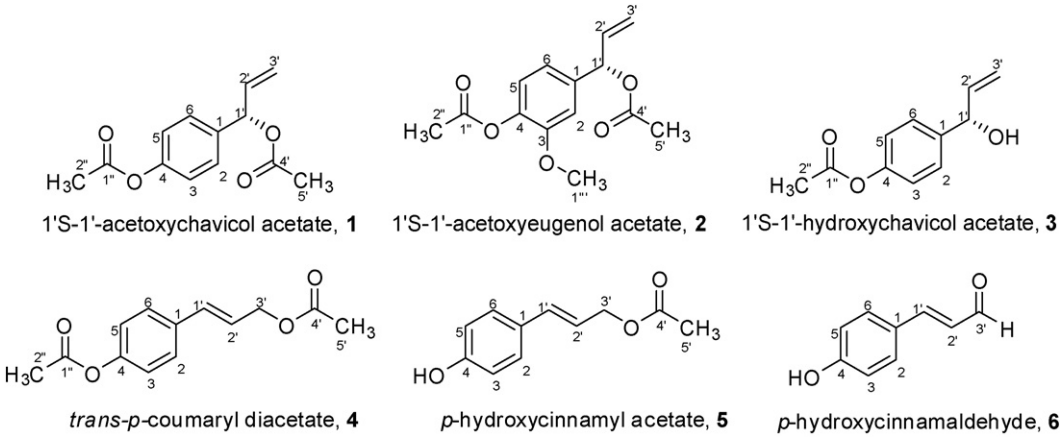

Fig. 1. Phenylpropanoids isolated from *Alpinia conchige*ra (Zingiberaceae).

specimen (KL5049) was deposited in the Herbarium of Chemistry Department, Faculty of Science, University Malaya.

Reagents

RPMI-1640, MEGM, fetal bovine serum (FBS) and antibiotics were purchased from Lonza Inc. (USA). MTT reagent, Annexin V-FITC/PI apoptosis detection kit, propidium iodide (PI), paclitaxel, RNase A and Suicide Track™ DNA Ladder Isolation Kit were purchased from EMD Chemicals Inc. (Calbiochem, San Diego, CA, USA).

Extraction and isolation natural compounds

Air-dried and powdered rhizomes of *Alpinia conchigera* (2.1 kg) were extracted with dichloromethane at room temperature (72 h). The solvent was evaporated *in vacuo* to give dichloromethane extract. The extract was subjected to column chromatography (CC) on silica gel (Merck Kiesegel 60) with stepwise gradient of hexane-ethyl acetate. Fractions were collected separately and concentrated *in vacuo* at 40 °C. Fractions with similar profiles in TLC were pooled together to obtain six subfractions which were then subjected to further chromatographic analysis which yielded six compounds (1–6). The structures of compounds 1–6 were determined based on comparison of the ^{1}H and ^{13}C NMR data with those reported in the literatures (Lee and Ando 2001; Ando et al. 2005; Janssen and Scheffer 1985; Yang and Eilerman 1999; Barik et al. 1987; Mitsui et al. 1976). All spectral data were obtained on the following instruments; IR on the PerkinElmer FT-IR spectrometer RX1, UV on a Shimadzu UV-160A UV-Visible Recording Spectrophotometer, NMR on a JEOL (Japan Electronic Optics Laboratory Co. Ltd., Tokyo, Japan) JNM-LA400 FT-NMR spectrometer system (400 MHz) and MS on a Shimadzu GC-MS spectrometer (HP 6890 Series Mass Selective Detector and HP 6890 Series GC System).

LCMS analysis

LCMS analysis was done using Shimadzu LCMS-IT-TOF (Columbia, MD, USA) with a binary pump, an automatic injector and

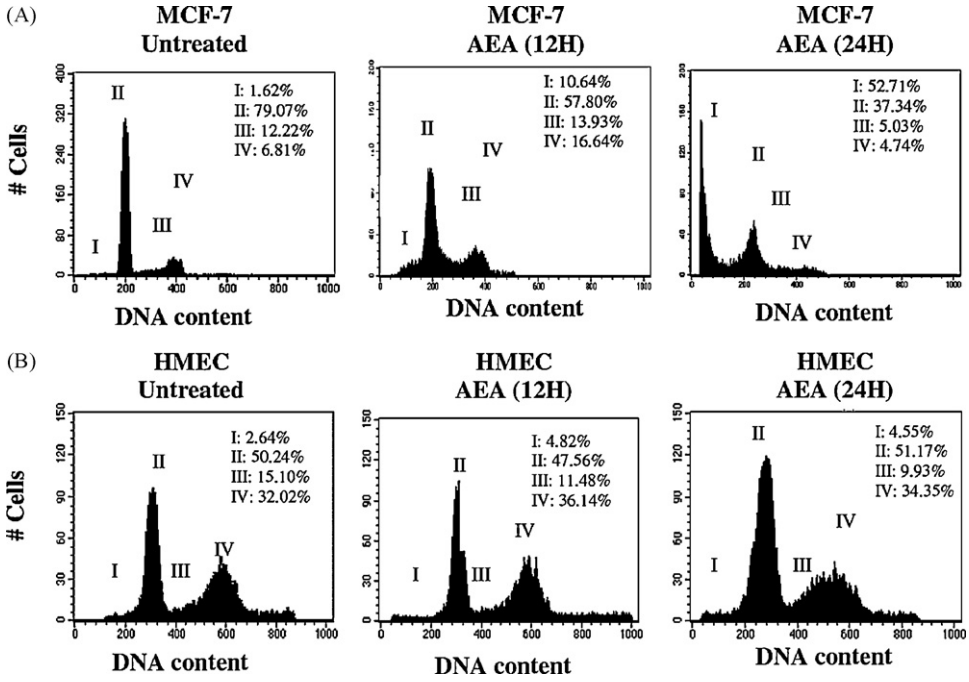

Fig. 2. Cell cycle distribution of MCF-7 and HMEC cells using flow cytometry after staining with propidium iodide (PI). (A) MCF-7 human breast cancer cells before and after AEA treatment for 12 and 24 h. (B) HMEC normal breast cell controls were treated with AEA for 12 and 24 h followed by cell cycle analysis. I: Sub-G_1; II: G_0/G_1; III: S; IV: G_2/M. All experiments are a representative of 20,000 cells and the percentage of cells in all cell cycle phases are indicated.