

Inhibitory effects of leaf extract of *Apama tomentosa* on ovarian cancer

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Abstract: Crude and hexane leaf extracts of *Apama tomentosa* (Aristolochiaceae) showed inhibitory effect on ovarian adenocarcinoma (CaOV-3) cells cultured in vitro with IC₅₀ of 46.13 ± 1.09 µg/ml and 18.50 ± 0.50 µg/ml respectively. The crude extract exerted a mild inhibitory effect on peripheral blood mononuclear cells (PBMCs). Hexane extract and palmitone did not exert any significant effect on the PBMC proliferation. Subsequent isolation of the leaf extract led to the isolation and identification of palmitone (16-hentriacontanone). This compound exhibited inhibitory effect (IC₅₀ = 17.70 ± 1.86 µg/ml) on CaOV-3 but was ineffective on PBMCs. This plant compound can potentially help to treat ovarian cancer.

Keywords: *Apama tomentosa*; Aristolochiaceae; palmitone; CaOV-3

INTRODUCTION

Apama tomentosa (Bl.) Engl. of Aristolochiaceae is known as 'hempedu beruang' in the Malay language. It is a shrub found in the secondary forests of Malaysia, India and Indonesia (Wiart, 2005). (Fig. 1). To date, there is only one published report on the antimicrobial activity of this plant (Wiart et al., 2004) and the anticancer properties are not known. The leaves of *A. tomentosa* are used traditionally by the aboriginals for treating inflammation, pain, snake-bite and gynaecological ailments (Mat So'ad and Yusoff, 2003).

Ovarian cancer is the fifth most common cancer among females in Malaysia (National Cancer Registry, 2003) after breast, cervix uteri, colon and corpus uteri cancer, constituting 4.1% of the female cancers, common in all age groups unlike breast and cervix cancer, seen at a certain age. If ovarian cancer is diagnosed late, the five-year survival rate is only 15% for patients in advanced stage (Breedlove and Busenhardt, 2005). This is due to complications such as metastasis of cancer cells to other parts of the body. The ovarian cancer cells are known to develop resistance against chemotherapeutic agents such as carboplatin (Österberg et al., 2005). Thus the search for new anticancer agents or adjuvant to the existing therapy will be useful in better management of the disease.

This paper discusses the effect of leaf extract of *A. tomentosa* on ovarian cancer cells and to isolate and identify the most potent, inhibitory compound.

MATERIALS AND METHODS

Instrumentation: Optical rotations were measured with Spectrum RX1 Perkin Elmer. NMR spectroscopic data were recorded at room temperature on JEOL JNM-LA400, FT-NMR System (400 MHz) with tetramethylsilane (TMS) as internal standard. Gas chromatography mass spectrometric analysis was performed with HP-6890 series as selective detector. The column used was HP5-MS coated with 5% phenyl methyl siloxane (30 m x 0.25 mm x 0.25 µm). The column temperature was programmed to increase from 60°C to 280°C at a rate of 10°C/minute and helium was the carrier gas. The mass of the compound was recorded at 280°C for 30 minutes. Analytical thin-layer chromatography (TLC) was performed on TLC aluminium sheets coated with silica gel 60 F₂₅₄.

Plant material: The leaves of *A. tomentosa* plant were collected from Templer's Park, Selangor (Malaysia) and the plant was identified by Dr. Christophe Wiart. A voucher specimen (code: C12L) is deposited at the Department of Pharmacy, Faculty of Medicine, University of Malaya.

Inhibition guided isolation: The air-dried powdered leaves of *A. tomentosa* (50 g) were exhaustively extracted with methanol (500 ml for three times) at 60°C and the rotary evaporated extract was labelled as crude extract. The same procedure was repeated using hexane. Subsequently, the rotary evaporated dried hexane extract was fractionated by silica gel 60 (2 x 50 cm) chromatography using gradient mixtures of hexane, ethyl acetate and methanol (95:5:0 – 0:5:95). Among all the fractions collected, hexane fraction tube 2 (H2) showed the highest inhibitory effect on CaOV-3 cells with the best yield (0.239mg,

47.78%). H₂ was further purified by repeatedly washing with hexane to yield a white powder compound (H₂P), identified as palmitone (16-hentriacontanone) using a GC-MS (m/z, %): 450 [M⁺] (calculated for C₃₁H₆₂O). This compound exhibited similar spectroscopic data (¹H and ¹³C NMR) corresponding to published values (Gonzalez-Trujano et al., 2001).

Determination of inhibitory effect: The crude extract, hexane extract and palmitone compound from *Apama tomentosa* were tested on ovarian adenocarcinoma cell line (CaOV-3) from American Type Culture Collection (ATCC) and human peripheral blood mononuclear cells (PBMCs) from healthy donors. The cytotoxic effect was determined using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay outlined by Mosmann (1983).

RESULTS AND DISCUSSION

The crude extract, hexane extract and palmitone (Figure 2) from *A. tomentosa* exerted inhibitory effect on the CaOV-3 cells (Table 1). Palmitone was found to be the major component of the hexane extract. The IC₅₀ of Palmitone and hexane against CaOV-3 cell proliferation was approximately the same (Table 1). The crude extract exerted a mild inhibitory effect on PBMCs, however hexane extract and palmitone did not exert any significant effect on the PBMC proliferation. (Figure 3).

Palmitone is often present on the surface of leaves as an epicuticular wax and is biosynthesized from long-chain fatty acids (Rhee et al., 1998). This wax is essential to protect leaves against ultra violet damage and water loss during drought season (Kolattukudy, 1980). In addition, it also serves as a defence layer to prevent bacterial (Sharma, 1993) and fungal attack.

Previously palmitone was reported for its antibacterial activity against *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus viridans*, *Escherichia coli*, *Pseudomonas pyocyanea* and *Klebsiella* (Sharma, 1993) and it showed anticonvulsant activity against epilepsy disorder (Gonzalez-Trujano et al., 2001). However, there appears to be no report on anticancer activity of palmitone against cancer cells.

It is noteworthy that the palmitone purified from *A. tomentosa* exerted the most potent inhibitory effect on CaOV-3 cells without having any cytotoxic effect on human PBMCs. In conclusion, palmitone was identified as the active inhibitory compound of the hexane extract of the leaves of *A. tomentosa*. This compound is, to our knowledge, the first aliphatic

ketone with antiproliferative property against ovarian adenocarcinoma cells which can represent a new chemical of natural origin that can potentially help treat ovarian cancer.

ACKNOWLEDGEMENTS

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Figure 1A: *Apama tomentosa*; leaf



Figure 1B: *Apama tomentosa*; part of plant

Figure 2. Structure of palmitone (C₃₁H₆₂O)

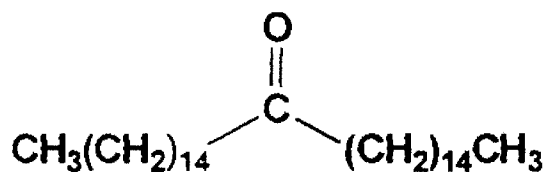
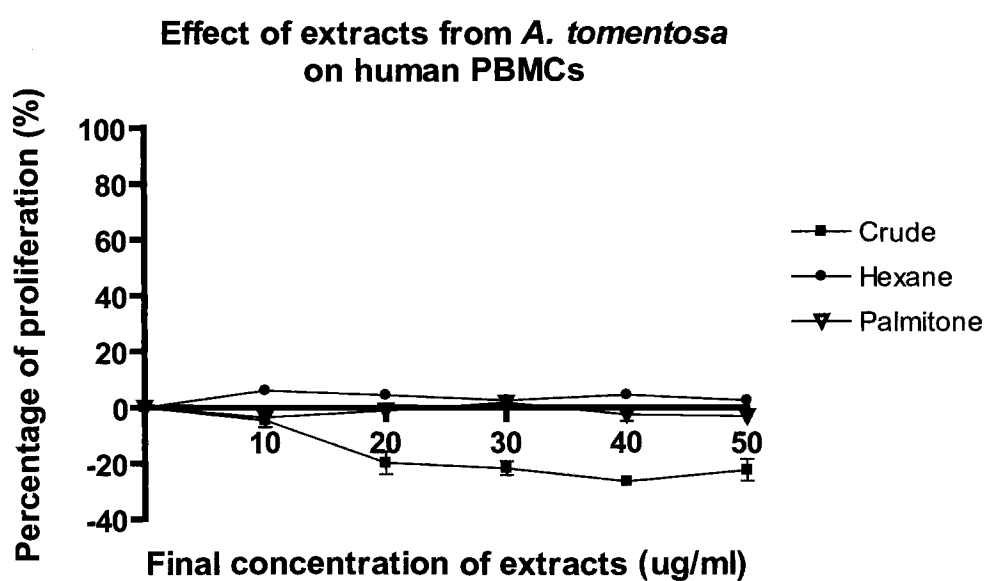


Figure 3. Dose response effect of extracts of *A. tomentosa* on human PBMCs proliferation



Human peripheral blood mononuclear cells (PBMCs) were treated with crude extract, hexane extract and palmitone from the leaves of *A. tomentosa* at concentration as shown. Percentage of proliferation (%) was measured by MTT assay. Error bars represent standard deviation (SD) of the mean calculated based on three separate experiments.

Table 1. Inhibitory effect of crude, hexane extract and palmitone obtained from *A. tomentosa* on CaOV-3 cells.

Extract/ compound	IC ₅₀ (µg/ml) ^a
Crude	46.13 ± 1.09
Hexane	18.50 ± 0.50
Palmitone	17.70 ± 1.86
Colchicine ^b	5.82 ± 0.14

^aResults are expressed as the concentration of extract/ compound that exerted 50% inhibition

^bPositive control substance