

# Artocarpin, A Promising Compound as Whitening Agent and Anti-skin Cancer

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## Abstract

In our search for natural products from wood on cosmetics and drugs purposes and on the basis of melanin biosynthesis assay guided fractionation, artocarpin was isolated from wood of Nangka (*Artocarpus heterophyllus*). To evaluate the potency as a whitening agent of artocarpin and its anti-skin cancer (cytotoxicity effect), the MTT assay was used to evaluate its cytotoxicity on cells and melanin biosynthesis assays was performed to determine its whitening agents potency. The evaluation of cytotoxicity on B16 melanoma cells of Artocarpin resulted the IC<sub>50</sub> was 10.3  $\mu$ M and melanin biosynthesis assay with IC<sub>50</sub> 6.7  $\mu$ M with out cytotoxicity. Based on the results, suggested that artocarpin have a potent to be developed as whitening agent and skin cancer drug.

**Key words:** B16 melanoma cell, whitening agent, cytotoxicity, artocarpin, *Artocarpus heterophyllus*.

## Introduction

Nangka or Jack fruit (*Artocarpus heterophyllus*) is well known in Indonesian as a fruit plant. The fruit is edible and have delicious taste. In addition, Khan *et al* (2003) reported that this plant has many functions especially for traditional medicine, for example the fruit and seed for tonic; root for diarrheae and fever; wood for muscle contraction; leave for activating milk in human and animal; antishyphilic, vermifuge; leaf ash for ulcers and wound.

In effort to find out a new material from natural resources for inhibiting biosynthesis of melanin in human skin (for whitening agent), some *Artocarpus* species have been screened. Based on those results, 9 of flavonoids and a stilbene compound have been isolated. This isolated compounds showed to inhibit melanin production in B16 melanoma cells culture (Arung *et al.* 2005; 2006a; 2006b). One of those compounds was artocarpin. The cytotoxicity test of artocarpin on B16 melanoma cells showed a positive effect, meaning that compounds inhibit the proliferation of the cells growth. Therefore, this paper will explain the function of artocarpin as a potential substance for whitening agent and anti-cancer drug.

## Material and Method

### Reagent

The chemicals and reagent used in this experiment were dimethylsulfoxide (DMSO), isopropanol (Wako, Japan), theophylline (Sigma-Aldrich Chemle GmbH, Steinheim, Germany), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA), arbutin, (Tokyo Kasei Kogyo, Tokyo, Japan), fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA), Eagle's minimal essential media (EMEM) (Nissui, Tokyo, Japan), trypsin (Invitrogen, Tokyo, Japan), HCL (Wako, Japan), and artocarpin (isolated from sapwood of *Artocarpus heterophyllus*).

**Cell Culture.** A mouse melanoma cell line, B16, was obtained from RIKEN Cell Bank. The cells were maintained in EMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 0.09 mg/ml theophylline. The cells were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>.

### Determination of Melanin Content and Cell Cytotoxicity in Mouse B16 Melanoma Cells.

The melanin biosynthesis inhibitory activity of isolated compounds on activated B16 melanoma cells by  $\alpha$ -MSH or Forskolin was determined as follows.

**Inhibitory Effect of Melanin Biosynthesis using Cultured B16 Melanoma Cells.** Confluent cultures of B16 melanoma cells were rinsed in phosphate-buffered saline (PBS) and removed from the plastic using 0.25% trypsin/EDTA. The cells were placed into 24-well plastic culture plates at a density of  $1 \times 10^5$  cells/well and incubated for 24 h in media prior to being treated with the samples. After 24 h, the media were replaced with 998  $\mu$ l of fresh media and 2  $\mu$ l of DMSO was added with or without (control) the test sample at various concentrations. The cells were incubated for an additional 48 h, and then the medium was replaced with fresh medium containing each sample. After 24 h, the remaining adherent cells were assayed (see below).

**Melanin Biosynthesis Inhibitory Activity of Isolated Compounds on Activated B16 Melanoma Cells by  $\alpha$ -MSH or Forskolin.** Confluent cultures of B16 melanoma cells were rinsed in phosphate-buffered saline (PBS) and removed from the plastic using 0.25% trypsin/EDTA. The cells were placed in two plates of 24-well plastic culture plates (1 plate is for determining of melanin and other is for cell viability) at a density of  $1 \times 10^5$  cells/well and incubated for 24 h in media prior to being treated with the samples. After 24 h, the media were replaced with 998  $\mu$ l of fresh media and 2  $\mu$ l of DMSO (control),  $\alpha$ -melanocyte

stimulating hormone ( $\alpha$ -MSH, 200 nM) or forskolin (20  $\mu$ M) and the test sample at various concentrations ( $n=3$ ). The cells were incubated for an additional 48 h, and then the medium was replaced with fresh medium containing each sample. After 24 h, the remaining adherent cells were assayed (see below).

**Determination of Melanin Content in B16 Melanoma Cells.** The melanin content of the cells after treatment was determined as follows. After removing the medium and washing the cells with PBS, the cell pellet was dissolved in 1.0 ml of 1N NaOH. The crude cell extracts were assayed using a micro plate reader (Bio-Tech, USA) at 405 nm to determine the melanin content. The results from the cells treated with  $\alpha$ -MSH or forskolin and the test samples were analyzed as a percentage of the results from the control culture.

#### Cell Viability

Cell viability was determined by use of the micro culture tetrazolium technique (MTT). The MTT assay provides a quantitative measure of the number of viable cells by determining the amount of formazan crystals produced by metabolic activity in treated versus control cells. Culture was initiated in 24-well plates at  $1 \times 10^5$  cells per well. After incubation, 50  $\mu$ l of MTT reagent [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide in PBS (5mgml<sup>-1</sup>)] was added to each well. The plates were incubated in a humidified atmosphere of 5% of CO<sub>2</sub> at 37°C for 4 h. After the medium was removed, formazan crystals were dissolved in 1.0ml of 0.04 N HCl, and the absorbance was measured at 570nm relative to 630nm. The data were analyzed using the two-tailed Student's t-test against the control. We also conducted cell counting to compare with MTT result.

### Results and Discussion

#### Inhibition of Melanin Biosynthesis for Skin Whitening Agent

Regarding to the results on melanin biosynthesis of artocarpin without or less cytotoxicity (Table 1), the melanin biosynthesis inhibitory in activated B16 melanoma cells was investigated. The experiment was focused on artocarpin (Figure 1).

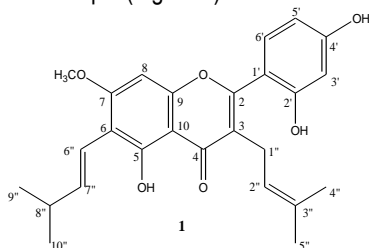


Figure 1. Structure of Artocarpin.

The effect of  $\alpha$ -MSH and cAMP on regulation of melanogenesis involves Microphthalmia Transcription Factor (MITF) as signal transducer. The effect of  $\alpha$ -MSH and cAMP could be explained as follow. First,  $\alpha$ -MSH is binded by melanocortin receptor (MC1R) which coupled to alfa G-protein. Then, alfa G-protein will activate AC (Aden late cyclise) resulting in significant increase of intracellular camp (cyclic AMP) content (Tsatmali *et al.* 2002). Second, the cAMP binds two sites of the regulatory subunit of protein kinase A (PKA), allowing the catalytic subunit to be liberated and activated. PKA is then able to phosphorylate its substrates, including ion channels, enzymes and regulatory proteins. PKA is also translocated to the nucleus where it phosphorylates the CREB (cAMP responsive element binding protein) family of transcription factors. Once phosphorylated, CREB proteins activate the expression of specific genes containing consensus CRE (cAMP responsive element) sequences in their promoters. Then, MITF is activated and binding to M box to up-regulated tyrosinase, TRP-1, DCT (dopachrome tautomerase or TRP-2) expression and thus, up-regulated melanin biosynthesis (Busca and Ballotti 2000).

Since  $\alpha$ -MSH and forskolin can activate the melanogenesis, B16 melanoma cells were activated with  $\alpha$ -MSH (200 nM) or forskolin (20  $\mu$ M). It is known that  $\alpha$ -MSH and forskolin were common and the best to characterize biologically activity of relevant agents inducing melanogenesis (Mun *et al.* 2004). The pigmentary effects of UV radiation (UVR) are mediated via melanocortin peptides such as  $\alpha$ -MSH. Keratinocytes and melanocytes secrete melanocortin peptides to response to UVR. Therefore,  $\alpha$ -MSH can act as paracrine and autocrine factor in the regulation of melanocytes and skin pigmentation (Tsatmali *et al.* 2002). Also, Thody (1999) reported that keratinocytes comprise a major source of peptides ( $\alpha$ -MSH and ACTH), although  $\alpha$ -MSH is present in higher concentrations in melanocytes. In addition, Bertolotto *et al.* (1996) and Price *et al.* (1998) reported that tyrosinase activity is strongly activated by  $\alpha$ -MSH and cAMP elevating agents (such as forskolin, cholera toxin, and isobutylmethylxanthine or IBMX) in B16 melanoma cells. These observations emphasized the crucial role of  $\alpha$ -MSH and cAMP in the regulation of melanogenesis.

The effect of artocarpin activated with  $\alpha$ -MSH or forskolin in B16 melanoma cells can be seen in Figure 2, respectively. As depicted in that figure, the melanin content of cells that activated with  $\alpha$ -MSH or forskolin without addition of the samples were higher than control (without  $\alpha$ -MSH or forskolin and the samples). The figure also indicated that artocarpin still inhibited melanin biosynthesis even the cells were stimulated or activated with  $\alpha$ -MSH or forskolin. The decreasing of melanin content in normal and activated cells was showed similar

tendency and it's seems depending on the concentration of samples.

These findings also indicated that isoprenoid-substituted compound, artocarpin, still reduced melanin production in B16 melanoma cells even treated with  $\alpha$ -MSH and forskolin as agent-induced melanogenesis in melanocytes. These results also confirmed that isoprenoid-substituted compounds might be blocked the  $\alpha$ -MSH signal or cAMP activated by forskolin signal in the cell line that down-regulated the melanin biosynthesis. These hypotheses still need further experiment to clarify the mechanism of isoprenoid-substituted compounds inhibit melanin production in B16 melanoma cells.

#### Cytotoxicity Effect of Artocarpin on B1 Melanoma Cell for Anti-cancer Drug

In Table 2, the cytotoxicity effect of artocarpin on B16 melanoma cells is presented. In that table, the  $IC_{50}$  (concentration that inhibit 50% of cell growth) of artocarpin was 10.3  $\mu$ M. The *isoprenoid* chain that attach

in flavonoid (prenylated flavonoid) could be the main reason that caused cytotoxicity effect on the cell. As mentioned by Barron and Ibrahim (1996) the *isoprenoid* chain in flavonoid may cause the cytotoxic effect, especially the position of *isoprenoid* chain in fungi. Meanwhile, some researchers also reported the cytotoxicity effect of some prenylated flavonoid, such as artelastoxanthone effective for Hep 3B (liver cancer cell), MCF-7 (breast cancer cell), and HT-29 (colon cancer cell), except A549 (lung cancer cell); artonol A effective for A549, MCF-7, and HT-29 cells, except Hep 3B cell (Ko *et al.* 2005). Cudraflavanone C for HCT-166 (colon cancer cell), SMMC-7721(liver cancer cell), and BGC-823 (Gastric cancer cell). Cudraflavanone A was effective for SMMC-7721, SGC-7901(Gastric cancer cell) and BCG-823 cells (Zou *et al.* 2005). Based on these results, the prenylated flavonoids were tending to cytotoxic on some cancers cell. The results here, and the references show that prenylated flavonoid have a potent as cancer drug for future.

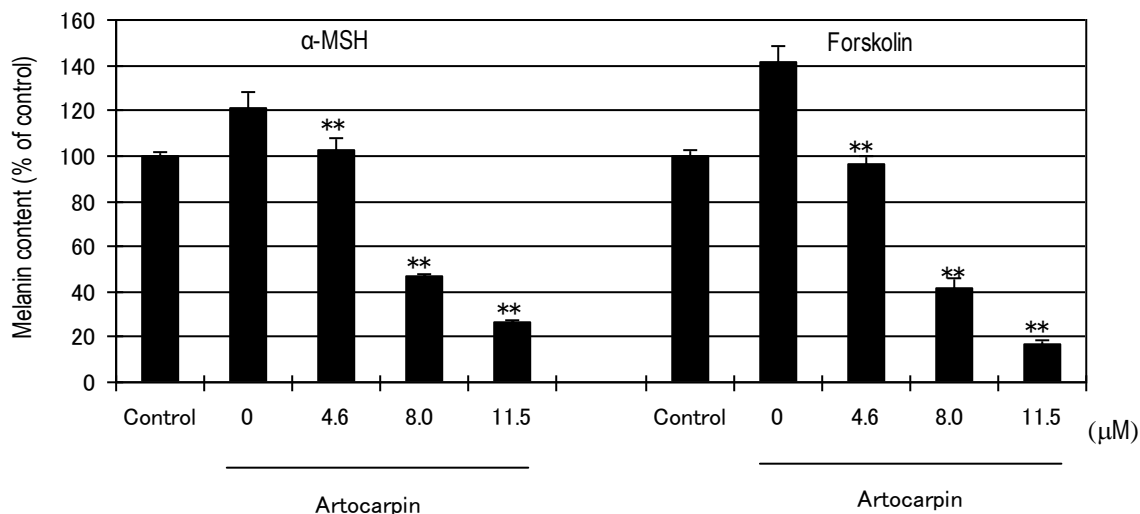


Figure 2. Effect of artocarpin (1) with  $\alpha$ -MSH (200 nM) or forskolin (20  $\mu$ M) in B16 melanoma cells. Data are represents the mean  $\pm$  S.D. (n=3). \*\*p<0.01 compared with value of  $\alpha$ -MSH alone or forskolin alone (Student's *t*-test).

Table 1. Effect of isolated compounds from sapwood of *Artocarpus heterophyllus* on melanin biosynthesis and cytotoxicity in B16 melanoma cells.

Compounds	Melanin inhibitor $IC_{50}$ ( $\mu$ M)	Cell viability (% vs control) <sup>a</sup>	Cytotoxicity $IC_{50}$ ( $\mu$ M)
Artocarpin (1) <sup>c</sup>	6.7	111*	10.3
Arbutin (positive control)	111	102*	-
Kojic acid (positive control)	>3,521 <sup>b</sup>	90 at 3,521 $\mu$ M*	-

Notes:

<sup>a</sup>: Cell viability (%) at the concentration of  $IC_{50}$  for melanin production in B16 melanoma cells

<sup>b</sup>: 40% inhibition of melanin production at 3,521  $\mu$ M.

<sup>c</sup>: Data were taken from Arung *et al.* (2006a)

\* Significant different from the control value: P<0.05

## Summary

Artocarpin not only showed inhibitory activity on melanin biosynthesis in normal B16 melanoma cell as well as suppressed the melanin content in activated of B16 melanoma cells by  $\alpha$ -MSH and forskolin as agents-induced melanogenesis.

Artocarpin also presented the property of cytotoxicity to B16 melanoma cell proliferation; it is a potent to be anti cancer drug.

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