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PRELIMINARY CYTOTOXIC EVALUATION OF *Andrographis paniculata* IN BREAST CANCER CELL LINES

Uji Pendahuluan Sitotoksik *Andrographis paniculata* pada Sel Kanker Payudara

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ABSTRAK

*Sambiloto (*Andrographis paniculata*) banyak digunakan untuk mengobati berbagai penyakit di Indonesia dan negara-negara Asia lainnya. Dalam studi ini, ekstrak metanol dan etanol sambiloto yang diperoleh dari B2PTO Tawangmangu telah diuji terhadap sel lini kanker payudara T47D dan MCF-7 dan sel lini normal fibroblast HFL-1 menggunakan reaksi enzimatik 3-(4,5-dimethylthiazoyl-2-yl) 2,5-diphenyltetrazoliumbromide (MTT). Uji in vitro terhadap sel lini normal fibroblast HFL-1 menunjukkan bahwa 50 ppm ekstrak metanol sambiloto tidak menghambat pertumbuhan sel. Tetapi, ekstrak metanol dan etanolnya menghasilkan IC_{50} yang relatif rendah pada sel lini kanker payudara, yaitu 111 ppm dan 122 ppm pada sel lini MCF-7 dan 70 ppm dan 197 ppm pada sel lini T47D. Selain itu, campuran ekstrak sambiloto yang mengandung 25% ekstrak* Thyponium divaricatum *dan* Anredera cordifolia *memberikan daya hambat pertumbuhan pada sel kanker payudara MCF-7 yang lebih besar, dengan nilai IC_{50} masing-masing adalah 68 ppm dan 34 ppm. Kesimpulannya, total ekstrak metanol atau etanol sambiloto yang diperoleh dari Tawangmangu memiliki potensi sebagai sumber senyawa anti-kanker serta perlu kajian lebih lanjut.*

Kata kunci: *Ekstrak* Andrographis paniculata*, MTT, sel lini normal, sel lini kanker, aktivitas anti kanker*

ABSTRACT

Sambiloto (*Andrographis paniculata*) is widely used as medicine to treat various diseases in Indonesia and other Asian countries. In this study, methanolic and ethanolic extracts of sambiloto collected from B2PTO Tawangmangu have been tested againts breast cancer cell lines of T47D and MCF-7 and normal fibroblast cell line of HFL-1 using enzymatic reaction of 3-(4,5-dimethylthiazoyl-2-yl) 2,5-diphenyltetrazoliumbromide (MTT). In vitro assay performed on normal fibroblast of HFL-1 cell line showed that 50 ppm of methanolic extract of sambiloto did not inhibit cell growth. However, methanolic and ethanolic extracts of sambiloto gave relatively low of IC_{50} on breast cancer cell lines which were 111 ppm and 122 ppm on the MCF-7 cell lines and 70 ppm and 197 ppm on the T47D cell lines, respectively. In addition, the mixture of sambiloto extract containing 25% of *Thyponium divaricatum* and *Anredera cordifolia* extracts confered greater growth inhibition on breast cancer cell line of MCF-7, where IC_{50} values were 68 ppm and 34 ppm, respectively. In conclusion, the total methanolic or ethanolic extract of sambiloto collected from Tawangmangu has potency as a source of anti-cancer compounds and needs further study.

Key words: *Andrographis paniculata* extract, MTT, normal cell line, cancer cell lines, anti-cancer activity

INTRODUCTION

The genus *Andrographis* is a widely distributed in South Asia and medicinally important member of Acanthaceae, consisting of approximately 40 species. *Andrographis paniculata* Nees (known as "Sambiloto**"** in Indonesia) is an herb well known in the South East Asian as traditional medicine and its beneficial actions are many and varied. Over the century, mostly the leaves and the roots have been used traditionally not only in Asia but also in Europe as folklore remedy for wide spectrum of ailments or supplements for health support as listed in Table 1 (Jarukamjorn and Nemoto 2008).

Phytochemical studies resulted in isolation of flavonoid and andrographolides from *A. paniculata* (Rao et al. 2004). Furthermore, three new compounds of *ent*-labdane diterpenoids, namely 19-norandrographolides A–C (compounds 1–3), were also isolated from the ethanolic extract of *A. paniculata*. (Zhang et al. 2006). Recent studies demonstrated that of *A. paniculata* has a potency as an antimicrobial activity (Sule et al. 2010). Previous research also reported that *A. paniculata* has anticancer and immunostimulatory effect (Kumar et al. 2004), anti-hyperglycemic and renal protective activities (Rao 2006).

A. paniculata are the most common traditional herbal that is used to achieve lower blood glucose in diabetic pasients by exhibits insulin-releasing actions (Wibudi et al. 2008). Hydroalcoholic extract of *A. paniculata* was reported as antioxidant, antilipid peroxidative and antiischemic activity and was used in ischemic heart diseases (Ojha 2009). In addition, leaf extract of *A. paniculata* has ability to suppress arsenic -provoked toxicity in human peripheral lymphocyte culture (Ghopalkrisnan and Rao 2008).

In this present study, we evaluated the methanolic and ethanolic extracts of *A. paniculata,* collected from Medicinal Plant Research Centre, Tawangmangu, Central Java, againts breast cancer cell lines of T47D and MCF-7 and normal fibroblast cell line of HFL-1 using enzymatic reaction of 3-(4,5-dimethylthiazoyl-2-yl) 2,5 diphenyl-tetrazolium bromide (MTT). In addition, we also tested the mixture extract of *A. paniculata* Ness with *Thyponium divaricatum* (known as Keladi tikus) as well as with *Anredera cordifolia* (known as Binahong).

Table 1. Traditional Uses of *A. paniculata*

Country	Native Names	Traditional Uses
Traditional Chinese Medicine (TCM)	Chuan-Xin-Lian	Fever, Common cold
	Chunlianqialio	Laryngitis, Pharyngitis, Tonsilitis
	Yiqianxi	Pneumonia
	Si-Fang-Lian	Respiratory infections
	Zhanshejian	Hepatitis
Traditional Indian Medicine	Kalmegh	Diabetes
	Kiryato	Dysentrery, Enteritis
	Maha-tikta	Helminth infection
	Bhunimba	Herpes, peptic ulcer, skin infection (topical use), snake- bites (topical uses)
Traditional Thai Medicine	Fah Thai Lai	Fever, Commond cold
	Nam Rai Pangpond	Non-infectious diarrhea
Malaysia	Hempedubumi	Diabetes
	Sambiloto	Hypertension
Japan	Senshinren	Fever, Common cold
Scandinavian	Green Chiretta	Fever, Common cold

MATERIALS AND METHODS

Reagents

Unless stated otherwise, all reagents used in this experiment were purchased from Sigma Aldrich (USA). RPMI-1640 medium, heat-inactivated fetal bofine serum (FBS), EDTA-trypsin was purchased from Gibco BRL (NY, USA). MTT powder (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Promega (USA).

Extract preparation

The aerial parts (stems and leaves) of *A. paniculata Nees*, *T. divaricatum* and leaves of *A. cordifolia* used in this study were collected from Medicinal Plant Research Centre, Tawangmangu, Central Java, Indonesia. The plant samples were dried at room temperature and finely powdered. Suitable amounts of the powdered materials were soaked in 95% ethanol or methanol (1 L per 100 g), and the solvent was evaporated at 40°C to dry up under reduced pressure using a rotary evaporator to produce crude extracts. The extract was then collected and stored at -20°C for further testing. The High Performance Liquid Chromatography (HPLC) analysis HPLC profile of *A. paniculata Ness* was carried out to detect the presence of active compound of Andrographolide (C18 volumn, λ = 223 nm, flowrate = 0.5-1 mL/min, 20 minutes).

Cell culture

The human breast adenocarcinoma cells (MCF-7 and T-47D) and the human normal lung fibroblast cell lines (HFL-1) were used to determine the cytotoxicity of the extract. Cells were cultured in T-flask containing RPMI 1640 medium supplemented with 10% heat inactivated foetal bovine serum (FBS), 50 IU/mL penicillin and 50 µg/mL streptomycin. The cells were maintained in the CO_2 incubator at 37ºC in a 5% CO_2 with 95% humidity. After reaching the confluency of approximately 70-80%, exponentially growing of the cells were washed twice with magnesium and calcium free phosphate buffer saline (PBS). The buffer solution was decanted and cells were detached with 0.025% trypsin-EDTA solution by incubating the cells at 37ºC in a 5% CO_2 with 95% humidity for couple of minutes, then cell culture growth medium were added to a volume of 5 mL. The cell suspension was then transferred to the falcon tube and the cell pellet was obtained by centrifugation (1000 g, 5 min). The cells were then resuspended in 10 mL of medium to make single cell suspension. The viable cells were counted by trypan blue exclusion assay in haemocytometer.

Cytotoxicity assay

Cells were seeded in 96-well plate in 100 µl of cell culture growth medium to a final concentration of $5x10^3$ cells/well and incubated to allow for cell attachment. After 24 h incubation, a partial monolayer was formed (confluency of 70-80%) then 100 µL of the medium containing the plant extract (initially dissolved in DMSO, those were: 250, 100, 50, 20 and 10 µg/mL) were added to the cells (normal and cancerous cell lines) and re-incubated for the next 24 h. After incubation with the plant extracts for overnight (24 hours), 100 µl of the media was aspirated and the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Promega, USA) solution (5 µg) in culture media of 100 µl was added. The incubation time for cells-MTT solution was 4 hours in which blue crystal were formed, then 100 µL of the stop solution (SDS 10%) were added and cells were then incubated further for the next 24 h. Reduced MTT was assayed at 550 nm using a microplate reader. Culture medium containing 0.1% DMSO was used as a solvent control and untreated cells were used as a negative control.

RESULTS AND DISCUSSION

High Performance Liquid Chromatography (HPLC) analysis of the ethanolic extract of *A. paniculata* Ness shows that the extracts containing the active compound of Andrographolide. The andrographolide concentration was various depend on the solvent composition. Apparently, the retention time (RT) was 2.422 minutes and the maximum concentration of andrographolide was achieved when sample was extracted using ethanol 70% (Figure 1). The andrographolide content was calculated based on the amount of extract being injected (1000 ppm) and the values are listed in Table 2.

Table 2. Andrographolide content of samples extracted using various solvent compositions

Solvent Composition	Area under curve	[Andrographolide, ppm]	% Andrographolide
96% ethanol	490398	15.97	1.6
Ethanol : H_2O = 70 : 30	3851355	96.25	9.6
Ethanol : H_2O = 50 : 50	1469925	69.85	7
H_2O (100%)	2032409	6.75	0.7

Note: Sample extract injected: 1000 ppm at λ= 223 nm

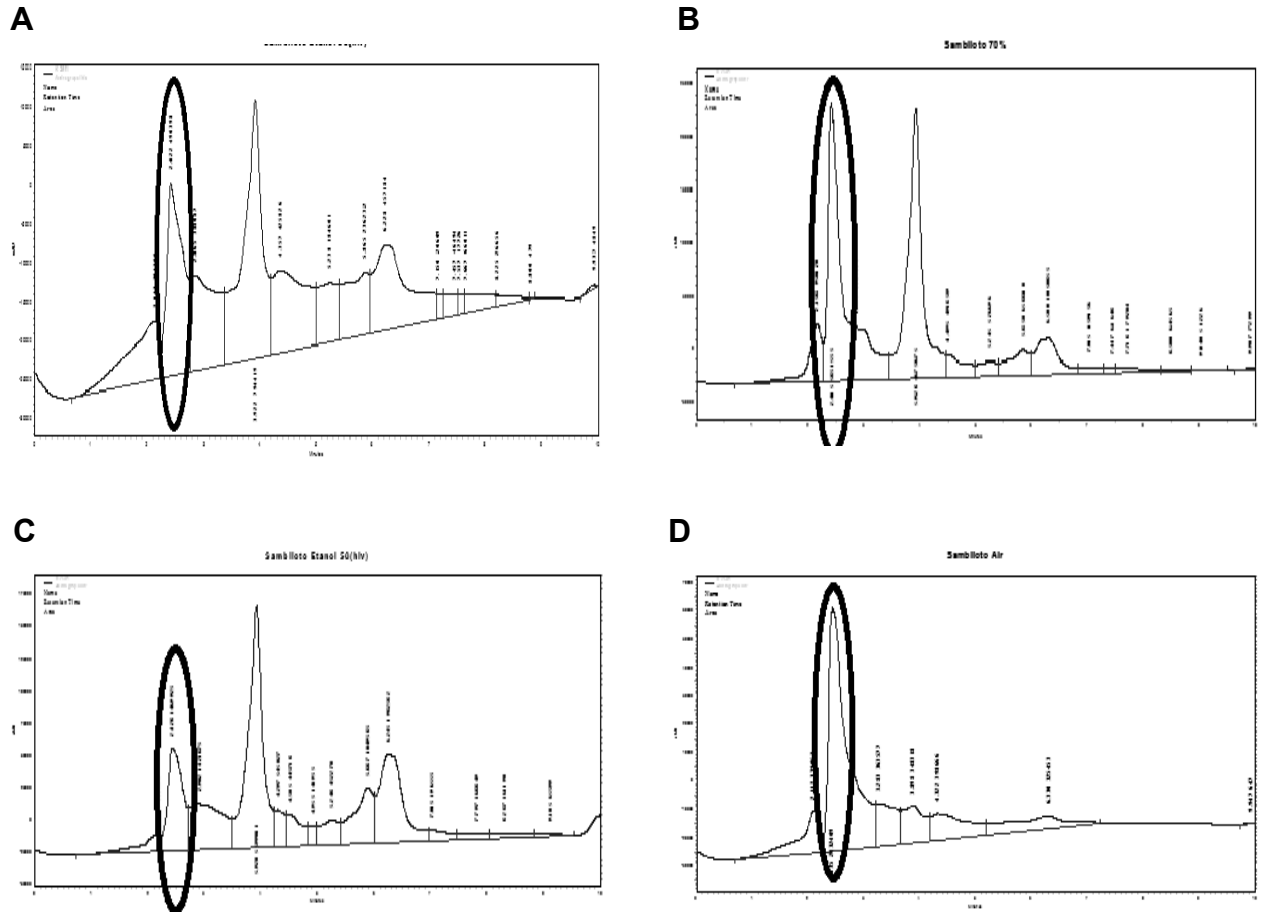

Figure 1. HPLC profile of *Andrographis paniculata Ness* extracts containing andrographolide; A. ethanolic extract of 96%, B. etahnolic extract of 70%, C. ethanolic extract of 50% and D. Decoction (H_2O extract)

In vitro cytotoxicity assay performed on normal fibroblast of HFL-1 cell line showed that 50 ppm of methanolic and ethanolic extract of *A. paniculata* did not inhibit cell growth, where the cell viability was about 101% on the methanolic extract and 94% on the methanolic extract. In this regards, we could simply assume that the extract has no cytotoxic effect on normal cell line of HFL-1. However, methanolic and ethanolic extracts of the sample gave IC_{50} relatively low on breast cancer cell lines of MCF7 which were 122 ppm and 111 ppm, respectively. The growth inhibition of the extract in MCF7 was dose dependent manner. As shown in Figure 2, the proliferative inhibition activity indicated that increasing extract concentration resulted in an increasing the percent proliferative inhibition. Furthermore, cytotoxicity of the methanolic and ethanolic extracts on other breast cancer cell line of T-47D showed an IC_{50} value of and 70 ppm and 197 ppm respectively. The proliferative inhibition

activity pattern of the extracts on T-47D cell line similar with the proliverative inhibition activity on MCF-7 cell line, done dependent manner, where increasing of the extract concentration treated followed with an increased the proliferative inhibition activity (Figure 3).

Our present study on *A. paniculata Ness* extract was in agrement with the results from previous researcher using another cell line, which reported that 10 ug/mL methanolic extract of *A. paniculata* inhibited proliferation of HT-29 (colon cancer cell) by 50%. In addition, the petroleum ether and dichlormethan extracts inhibited the proliferation of HT-29 cell with IC_{50} value of 46 ug/mL and 10ug/mL, respectively (Kumar et al. 2004). Furthemore, from the ethanolic extract of the aerial parts of *A. paniculata* resulted in the isolation of 14 compounds including flavonoids and labdane diterpenoids, where the compound 6 *was* rich source for the active compound of andrographolide. The bioactivity assays showed that metabolites 1-4 and 6-8 exhibited moderate cytotoxic activity against Jurkat, PC-3 and Colon 205 cell lines, where compound 6 had IC_{50} values of 0.05, 0.07 and 0.05 mm, respectively. Further, among these effective compounds, 3 and 6 selectively blocked the cell cycle progression at G0/G1, while 1, 2, 4, 7 and 8 metabolites blocked the same at G2/M phase of the Jurkat cell line (Geethangili et al. 2008). Subsequently, the mixture of 75% *A. paniculata* extract with 25% of *T. divaricatum* confered greater proliferative inhibition on MCF-7 cell with gave IC_{50} values of 68 ppm. Moreover, the combination of 75% *A. paniculata* extract with 25% *A. cordifolia* extracts resulted in lowest IC_{50} value of 34 ppm. The pattern of proliverative inhibition of the mixture of *A. paniculata* with *T. divaricatum* and *A. cordifolia* was shown on Figure 4. This result revealed possibility any synergistic action between the constituents resulted from the *A. paniculata* and from the *T. divaricatum* or the *A. cordifolia.* This result was also supported with previous study which reported that several fractions of the hexane and dichloromethane extracts of *T. divaricatum* were found to inhibit the growth of NCI-H23 non-small cell lung carcinoma cell line significantly, with IC_{50}<15µg/mL, and several fractions from this extract were also found to inhibit the growth of non-tumorigenic BALB/c 3T3 mouse fibroblast cell line. This particular fraction was not only less cytotoxic to the non-tumorigenic cells, where the IC_{50} was 48.6 µg/mL compared to IC_{50} 7.5 µg/mL for NCI-H23, but it was also found to induce apoptosis in the cancer cell line. GC–MS analysis revealed that D/F21 contains hexadecanoic acid, 1-hexadecene, phytol and a derivative of phytol (Lai et al. 2008). Subsequent studies reported that purification of the chemical constituents was guided by the antiproliferative activity using MTT. reagent on NCI-H23 (lung cancer) and HS578T (breast cancer) cell lines. Four pheophorbide related compounds, namely pheophorbide-a, pheophorbide-a′, pyropheophorbide-a and methyl pyropheophorbide-a were identified in the most active fraction, D/F19. These constituents exhibited

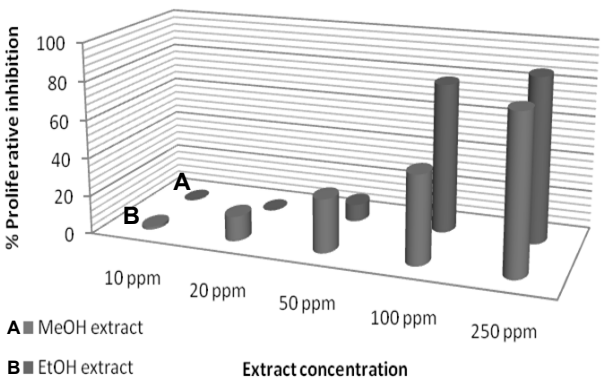

Figure 2. Effect of *A. paniculata* methanolic and ethanolic extract on proliferation of MCF-7 cancer cell line

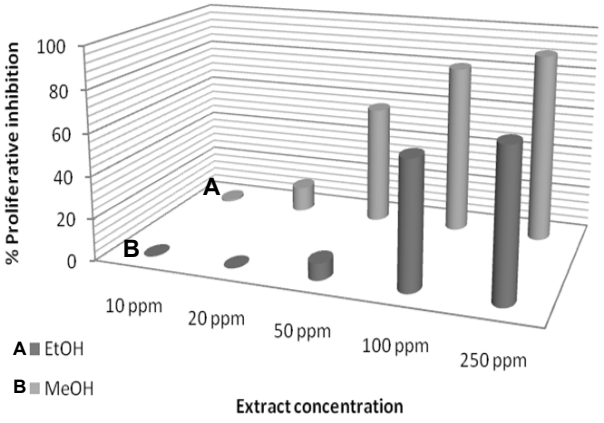

Figure 3. Effect of *A. paniculata* methanolic and ethanolic extract on proliferation of T-47D cancer cell line

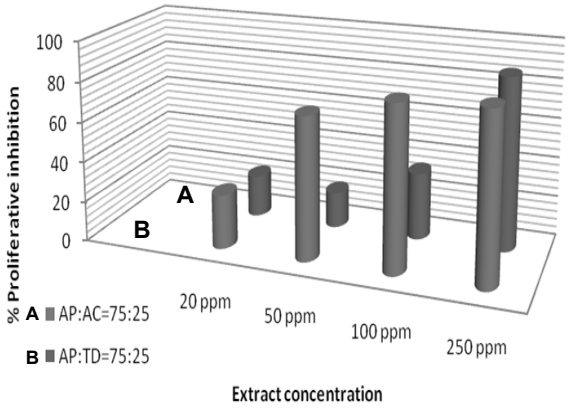

Figure 4. Effect of the mixture of *A. paniculata* with *T. divaricatum* and *A. cordifolia* ethanolic extract on proliferation of MCF-7 cancer cell line. AP : *A. paniculata*, AC: Anredera cordifolia, TD: *T. divaricatum*

antiproliferative activity against cancer cells and the activity increased following photoactivation. The inhibitory effect of the fractions was apoptotic in the absence of light. Other chemical constituents that have been identified in this study include hexadecanoic acid, oleic acid, linoleic acid, linolenic acid, campesterol, stigmasterol and β-sitosterol (Lai et al. 2010).

CONCLUSIONS

The total methanolic or ethanolic extract of medicinal plant *A. paniculata* collected from Tawangmangu, Central Java have potency as a source of anti cancer compounds and need to be further studied to understand the mechanism of action of the extracts against cancer cell.

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