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P-Glycoprotein Inhibitory Activity of Indonesian Medicinal Plants in Human Breast Cancer Cells

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Abstract – In order to examine their effects on the P-glycoprotein (P-gp) activity in human breast cancer cells, MCF-7/ADR, one hundred Indonesian plant extracts were screened. Among them, the five chloroform extracts of *Calotropis gigantea*, *Curcuma aeruginosa*, *Merremia mammosa*, *Sindora sumatrana*, and *Zingiber cassumunar*, showed the most potent P-gp inhibitory activity. When each of these extracts was treated together with the anticancer agent, daunomycin, they increased the cytotoxic activity of daunomycin up to IC_{50} values of less than 6.62 μM, which is a value with a positive control, verapamil. Also, other 15 plant extracts exhibited significant P-gp inhibitory activity with IC_{50} values between 6.62 and 13.20 μM. These prospective samples will be subjected to further laboratory phytochemical investigation to find active principles.
Keywords – P-glycoprotem, Indonesian plants, MCF-7/ADR cell, daunomycin

Introduction

Cancer chemotherapy has limited success due to the intrinsic or acquired resistance of cancer cells to a wide range of chemically and functionally diverse anticancer drugs, a phenomenon termed multidrug resistance (MDR). MDR is, at least in part, conferred by the over-expression of P-glycoprotein (P-gp) in the cell membrane, which acts as an energy-dependent drug efflux pump, resulting in decreased intracellular drug accumulation (Endicott *et al.*, 1989; Fardel *et al.*, 1996; Gottesman *et al.*, 1993). Many studies have shown that compounds found in fruits, vegetables, and plant-derived beverages such as tea and red wine, have not only anti-carcinogenic activities but may also modulate P-gp activity (Chieli *et al*, 1995; Christensen *et al*, 1996; Critchfield *et al*, 1994; Go *et al*, 2003; Phang *et al.*, 1993; Plouzek *et al.*, 1999).

In the present study, one hundred plant extracts that did not exhibit potent cytotoxicity, were tested to investigate their effects on P-gp activity in a human breast cancer cells, MCF-7/ADR.

Experimental

Plant materials and extractions – The Indonesian plants as test samples were collected in Surabaya, Indonesia, in 2001, and were identified by professor Tri Windono (University of Surabaya, Indonesia). The voucher specimens have been deposited at University of Surabaya. 500 g of each dried plant was ground and extracted with methanol by percolation. The filtered methanol extracts were evaporated under vacuum. The aqueous methanol extract was partitioned with *n*-hexane, chloroform, and *n*-butanol, subsequently.

Chemicals – Trichloroacetic acid (TCA), daunomycin (DNM), Hank's balanced salts without sodium bicarbonate (HBSS), verapamil, dimethyl sulphoxide (DMSO) and sulforhodamine B (SRB) were purchased from Sigma-Aldrich (St. Louis, MO,USA). Dulbecco's modified eagle medium/low glucose (DMEM), Trypsin-EDTA (0.25% trypsin-1 mM EDTA) and Penicillin-Strepto mycin were from Invitrogen (Calsbad, CA,USA). Fetal bovine serum (FBS) was obtained from Hyclone (South Logan, UT,USA).

Evaluation of inhibitory effects against the P-gp activity – Approximately 5000 MCF-7/ADR cells per well were seeded in 96 well tissue culture plates and allowed to attach for 24 hours at 37°C. Then, additional medium was added to each well containing the desired final concentration of daunomycin (9×10^{-8} M~7.2×10^{-5} M) in the presence and absence of plant extracts (50 μg/ml). Verapamil (50 μg/ml), a well-known P-gp inhibitor, was used in the study as a positive control. After a two hour

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exposure to daunomycin ± plant extracts, the cells were washed twice with HBSS and fresh medium was added to each well. The cells were allowed to grow for 72 hours (3 days) following which the total protein was measured using a SRB staining assay (Skehan *et al.*, 1990). Briefly, cells were fixed with 10% TCA for an hour, then washed with water 5 times and air-dried. SRB (0.4% w/v in 1% acetic acid) was added to each well for 30 minutes, followed by 4 washes with 1% acetic acid. After drying the plates, protein bound dye was dissolved in 10 mM Tris base (pH 10.0) and the absorbance of each well at 515 nm was measured using an ELISA plate reader. IC_{50} values were calculated using non-linear regression analyses (percent survival vs. DNM concentration).

Results and Discussion

One hundred Indonesian plant extracts were screened to investigate P-gp inhibitory activity in a human breast cancer cells, MCF-7/ADR. MCF-7/ADR cells are doxorubicin-resistant subline of the human breast cell line, MCF-7 and exhibited marked multidrug resistance, which was due to, at least in part, over-expression of P-gp (Fairchild *et al*, 1990). Daunomycin uptake in the MCF-7/ADR cells was significantly decreased by about 15% compared to the sensitive MCF-7 cells, confirming over-expression of P-gp (unpublished data). As judged in the criteria of P-gp inhibitory activity with IC_{50} of daunomycin < 13.2 μM in MCF-7/ADR cells, twenty extracts were evaluated as

Table 1. IC_{50} values of daunomycin in MCF-7/ADR cells after 2 hour incubation with plant extracts

Plant name and Authority	Family	Sample code[a]	Part used	IC_{50} (μM)
Acalypha indica L.	Euphorbiaceae	EA215H	Aerial parts	-
		EA215C		-
		EA215B		38.4
		EA215Aq		37.5
Ageratum conyzoides L.	Asteraceae	EA223H	Whole plants	-
		EA223C		-
		EA223B		12.3
		EA223Aq		18.1
Alpinia galanga (L.) Swartz.	Zingiberaceae	EA205H	Rhizome	-
		EA205C		-
		EA205B		-
		EA205Aq		33.2
Alstonia scholaris (L.) R. Br.	Apocynaceae	EA210H	Cortex	19.4
		EA210C		19.2
		EA210B		21.1
		EA210Aq		29.3
Amorphophallus campanulatus (Roxb) BI.Ex Decne	Araceae	EA218H	Tubera	21.3
		EA218C		23.2
		EA218B		45.4
		EA218Aq		33.8
Artocarpus communis Forst.	Moraceae	EA201H	Heart wood	15.2
		EA201C	(Lignum)	-
		EA201B		12.2
		EA201Aq		34.0
Azadirachta indica A. Juss.	Meliaceae	EA200H	Leaves	7.65
		EA200C		-
		EA200B		13.0
		EA200Aq		21.6
Calotropis gigantea (Wild.)Dryand. Ex W.T.Ait.	Asclepiadaceae	EA219H	Underground	18.9
		EA219C	parts(Root)	4.15
		EA219B		40.4
		EA219Aq		27.0
Cassia siamea Lamk.	Caesalpiniaceae	EA206H	Leaves	22.3
		EA206C		29.0
		EA206B		-
		EA206Aq		37.8
Colocasia esculenta (L.) Schott.	Araceae	EA199H	Corm	29.4
		EA199C		27.8
		EA199B		10.5
		EA199Aq		21.2
Curcuma aeruginosa Roxb	Zingiberaceae	EA195H	Rhizome	6.71
		EA195C		2.75
		EA195B		26.7
		EA195Aq		24.7
Curcuma heyneana Val. & v.Zijp	Zingiberaceae	EA196H	Rhizome	14.5
		EA196C		7.19
		EA196B		37.2
		EA196Aq		24.2

Table 1. Continued

Plant name and Authority	Family	Sample code[a]	Part used	IC_{50} (μM)
Dioscorea hispida Dennst.	Dioscoreaceae	EA220H	Tubera	43.3
		EA220C		24.5
		EA220B		54.7
		EA220Aq		44.2
Eclipta alba (L.) Hassk.	Asteraceae	EA214H	Aerial parts	25.1
		EA214C		-
		EA214B		40.4
		EA214Aq		30.9
Elephantopus scaber L.	Asteraceae	EA202H	Aerial part	-
		EA202C		-
		EA202B		22.8
		EA202Aq		29.8
Euphorbia prostata W. Ait.	Euphorbiaceae	EA213H	Whole plants	24.1
		EA213C		35.9
		EA213B		26.9
		EA213Aq		31.4
Excoecaria cochinchinensis Lour.	Euphorbiaceae	EA216H	Leaves	-
		EA216C		-
		EA216B		23.0
		EA216Aq		25.7
Justicia gendarussa Burm. F.	Acanthaceae	EA207H	Leaves	63.1
		EA207C		33.2
		EA207B		31.8
		EA207Aq		32.5
Kaempferia rotunda L.	Zingiberaceae	EA209H	Rhizome	22.4
		EA209C		31.4
		EA209B		36.3
		EA209Aq		43.1
Merremia mammosa (Lour.) Hallier F.	Convolvulaceae	EA211H	Tubera	-
		EA211C		3.58
		EA211B		62.7
		EA211Aq		46.3
Parameria laevigata (Juss.) Moldenke	Apocynaceae	EA224H	Cortex	34.1
		EA224C		20.5
		EA224B		29.5
		EA224Aq		45.6
Ruellia tuberosa L.	Acanthaceae	EA222H	Aerial parts	16.7
		EA222C		11.8
		EA222B		58.2
		EA222Aq		58.0
Sindora sumatrana Miq.	Caesalpiniaceae	EA221H	Fructus	8.77
		EA221C		6.23
		EA221B		31.1
		EA221Aq		28.5
Strychnos ligustrina Bl.	Loganiaceae	EA208H	Lignum	13.2
		EA208C		19.8
		EA208B		38.4
		EA208Aq		40.8
Tinospora tuberculata Beumee	Menispermaceae	EA203H	Caulis	11.4
		EA203C		12.3
		EA203B		16.1
		EA203Aq		22.6
Vernonia cinerea (L.) Less.	Asteraceae	EA212H	Aerial parts	68.6
		EA212C		19.1
		EA212B		43.7
		EA212Aq		29.9
Zingiber cassumunar	Zingiberaceae	EA204H	Rhizome	7.09
		EA204C		6.56
		EA204B		30.3
		EA204Aq		27.8
Zingiber zerumbet(L.) J.E.Smith.	Zingiberaceae	EA198H	Rhizome	9.32
		EA198C		9.48
		EA198B		30.6
		EA198Aq		32.7

[a]Sample code : H (hexane), C (chloroform), B (butanol), Aq (aqueous).

Daunomycin — 33.6 ± 2.99(n = 10)

Daunomycin + Verapamil (P-gp inhibitor) — 6.62 ± 1.89(n = 10)

* - : Marked cytotoxicity was found at 50 g/ml of each plant extract.

active samples. Among them, the $CHCl_3$ extracts of *Calotropis gigantea*, *Curcuma aeruginosa*, *Merremia mammosa*, *Sindora sumatrana*, and *Zingiber cassumunar*, decreased the IC_{50} value of daunomycin to 4.15, 2.75, 3.58, 6.23 and 6.56 μM, respectively, indicating that these extracts had potent P-gp inhibitory activity. The IC_{50} value of daunomycin treated with verapamil was 6.62 ± 1.89 μM, suggesting that the five extracts were more potent than the positive control, verapamil. Moreover, these samples were plant extracts including mixtures of natural compounds, thus, there will be high possibility to isolate more potent lead compounds from these extracts in our future phytochemical study.

Other 15 plant extracts showed significant P-gp inhibitory activity with the daunomycin IC_{50} values between 6.62 and 13.2 μM. Although they exhibited less P-gp inhibitory activity than the positive control, they are evaluated to be quite strongly active because the positive control was a single compound whereas the tested samples were plant extracts, mixtures of various compounds. (Table 1)

On the basis of these results, further phytochemical study will be performed to isolate a potent P-gp inhibitor from the active plant extracts.

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