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Evaluation of Cytotoxic Potential of Indonesian Medicinal Plants in Cultured Human Cancer Cells

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Abstract – One-hundred and twenty plant extracts were prepared from 29 Indonesian plants and were primarily tested in vitro cytotoxicity in cultured human lung (A549), colon (Col2), and stomach (SNU-638) cancer cells. As a result, the 23 extracts were found to be active in the criteria of ED_{50}<20 μg/ml. Remarkable cytotoxicity was observed for chloroform and n-butanol extracts of *Calotropis gigantea*, with ED_{50} values ranging from 0.25 to 0.46 μg/ml. Five extracts derived from *Eclipta alba* and *Excoecaria cochinchinensis* displayed potent cell-line selective cytotoxicity, while the rest of 15 extracts showed modest cytotoxic activity against all of three cancer cells. In addition, the cytotoxic potential of subfractions of *Zingiber cassumunar* against a panel of human cancer cell lines is presented.

Key words – Cytotoxicity, Indonesian plants, Plant extracts, *Zingiber cassumunar*

Introduction

During the course of an ongoing collaborative research program on the investigation of the plant kingdom for novel potential antitumor agents, it was found that several fractions obtained from the 29 plants collected in Indonesia showed considerable activity in our standard cytotoxicity assay.

We have been interested in the ginger species as a new source of antitumor agents because most ginger rhizomes have been widely used in indigenous medicine for the treatment of inflammatory and other diseases in tropical areas (Larson *et al.*, 1988; Jitoe *et al.*, 1992; Ishida *et al.*, 2002). Many secondary metabolites such as curcuminoids (Ruby *et al.*, 1995; Ishida *et al.*, 2002; Vimala *et al.*, 1999), flavonoid glycosides (Nakatani *et al.* 1991; Murakami *et al.* 1992), sesquiterpenes (Murakami *et al.* 2002), polyphenols (Murakami *et al.* 1992) have been reported from various tropical gingers.

In the present investigation, different extracts (n-hexane, chloroform, n-butanol, water) of 29 Indonesian plants were screened for their cytotoxicity in three human cancer cell lines including A549, Col2, and SNU638. Furthermore, bioassay-guided fractionations with chloroform-soluble fraction of one of active plants, *Z. cassumunar,* were performed, and the first column fractions were tested for their cytotoxicity.

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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. 500g 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. Flash column chromatography of the $CHCl_3$ extract of *Z. cassumunar* was carried out on Si gel 60 (230-400 mesh, Merck, Germany) with mild nitrogen pressure. Column chromatography was monitored by TLC (Si gel 60 F_{254} plates, 0.25 mm thickness) with visualization under UV light (254 and 365 nm) and spray of sulfuric acid solution (10 v/v% in ethanol) followed by heating (5 min in 135°C).

Chemicals – All chemicals and reagents used were of highest purity. Trichloroacetic acid (TCA), and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (St. Louis, MO). Minimal essential medium with Earles salt (MEME), fetal bovine serum (FBS), non-essential amino acid solution (10 mM, 100X), trypsin-EDTA solution (1X) and antibiotic-antimycotic solution (PSF) were from GIBCO-BRL (Grand Island, NY).

Evaluation of cytotoxic potential with human cancer cell lines – Cytotoxic potential was determined as described

previously (Lee *et al.*, 1998). Briefly, cells (in log growth phase) were counted, diluted to 5×10^4 cells/ml with fresh medium, and added to 96-well microtiter plates (190 μl/well) containing test materials (10 μl in 10% aqueous DMSO). Test plates were incubated for 3 days at 37°C in CO_2 incubator. For zero day controls, cells were incubated for 30 min at 37°C in CO_2 incubator. All treatments were performed in triplicate. After the incubation periods, cells were fixed by the addition of 50 μl of cold 50% aqueous trichloroacetic acid (4°C for 30 min), washed 4-5 times with trap water, and air-dried. The fixed cells were stained with sulforhodamin B (SRB) (0.4% w/v SRB in 1% aqueous acetic acid) for 30 min. Free SRB solution was then removed by rinsing with 1% acetic acid. The plates were then air-dried, the bound dye was solubilized with 200 μl of 10 mM tris-base (pH 10.0), and absorbance was determined at 515 nm using an ELISA plate reader. Finally, the absorbance values obtained with each of the treatment procedures were averaged, and the averaged value obtained with the zero day control was subtracted. These results were expressed as a percentage, relative to solvent-treated control incubations, and ED_{50} values were calculated using non-liner regression analyses (percent survival versus concentration).

Results and Discussion

One-hundred and twenty Indonesian plant extracts were screened to evaluate the cytotoxicity in three human cancer cell lines including lung (A549), colon (Col2), and stomach (SNU638). As judged in the criteria of cytotoxicity activity with ED_{50}<20 μg/ml, twenty-three extracts were found to be active as shown in Table 1. Among them, fifteen extracts exhibited cytotoxic activity against A549, the 13 extracts showed cytotoxicity against Col2, and the 11 extracts were cytotoxic against SNU638. Chloroform and n-butanol extracts of *Calotropis gigantea* demonstrated the most significant cytotoxicity against all three cancer cells with ED_{50} values ranging from 0.25 to 0.46 μg/ml, which are comparable to the positive control, ellipticine (ED_{50} = ~0.3 μg/ml). The chloroform-soluble fraction of *Elephantopus scaber* also exhibited potent cytotoxic activity, with ED_{50} values of 3.24, 3.07 and 1.69 μg/ml, respectively, against the test three cell

Table 1. Cytotoxic potential of Indonesian plants on human cancer cells

Plant name and Authority	Family	Smple code[a]	Part used	A549[b]	Col2[c]	SNU638[d]
Acalypha indica L.	Euphorbiaceae	EA215H	Aerial parts	>20	16.772	>20
		EA215C		>20	>20	>20
		EA215B		>20	>20	>20
		EA215Aq		>20	>20	>20
Ageratum conyzoides L.	Asteraceae	EA223H	Whole plants	>20	>20	>20
		EA223C		>20	16.89	16.5
		EA223B		>20	>20	>20
		EA223Aq		>20	>20	>20
Alpinia galanga (L.) Swartz.	Zingiberaceae	EA205H	Rhizome	15.09	11.12	>20
		EA205C		12.69	5.61	1.27
		EA205B		>20	>20	>20
		EA205Aq		>20	>20	>20
Alstonia scholaris (L.) R. Br.	Apocynaceae	EA210H	Cortex	>20	>20	>20
		EA210C		>20	>20	>20
		EA210B		>20	>20	>20
		EA210Aq		>20	>20	>20
Amorphophallus campanulatus (Roxb) BI.Ex Decne	Araceae	EA218H	Tubera	>20	>20	>20
		EA218C		>20	>20	>20
		EA218B		>20	>20	>20
		EA218Aq		>20	>20	>20
Artocarpus communis Forst.	Moraceae	EA201H	Heart wood	>20	>20	>20
		EA201C	(Lignum)	15.13	15.31	12.59
		EA201B		>20	15.57	>20
		EA201Aq		>20	>20	>20
Azadirachta indica A. Juss.	Meliaceae	EA200H	Leaves	>20	>20	>20
		EA200C		>20	>20	9.35
		EA200B		>20	>20	>20
		EA200Aq		>20	>20	>20

Table 1. Continued

Plant name and Authority	Family	Smple code[a]	Part used	A549[b]	Col2[c]	SNU638[d]
Calotropis gigantea (Wild.) Dryand. Ex W.T.Ait.	Asclepiadaceae	EA219H	Underground	15.1	18.69	18.58
		EA219C	parts(Root)	0.29	0.37	0.43
		EA219B		0.25	0.42	0.46
		EA219Aq		8.13	19.57	16.93
Cassia siamea Lamk.	Caesalpiniaceae	EA206H	Leaves	>20	17.55	>20
		EA206C		>20	15.45	>20
		EA206B		>20	>20	>20
		EA206Aq		>20	>20	>20
Colocasia esculenta (L.) Schott.	Araceae	EA199H	Corm	>20	>20	>20
		EA199C		>20	>20	>20
		EA199B		>20	>20	>20
		EA199Aq		>20	>20	>20
Curcuma aerusinosa Roxb	Zingiberaceae	EA195H	Rhizome	>20	>20	>20
		EA195C		>20	>20	>20
		EA195B		>20	>20	>20
		EA195Aq		>20	>20	>20
Curcuma heyneana Val. & v.Zijp	Zingiberaceae	EA196H	Rhizome	>20	>20	>20
		EA196C		>20	>20	>20
		EA196B		>20	>20	>20
		EA196Aq		>20	>20	>20
Curcuma mangga Val. Et zijp.	Zingiberaceae	EA197H	Rhizome	16.98	15.71	8.15
		EA197C		>20	17.56	15.36
		EA197B		>20	>20	>20
		EA197Aq		>20	>20	>20
Dioscorea hispida Dennst.	Dioscoreaceae	EA220H	Tubera	>20	>20	>20
		EA220C		>20	>20	>20
		EA220B		>20	>20	>20
		EA220Aq		>20	>20	>20
Eclipta alba (L.) Hassk.	Asteraceae	EA214H	Aerial parts	>20	5.87	>20
		EA214C		>20	>20	>20
		EA214B		>20	>20	>20
		EA214Aq		>20	>20	>20
Elephantopus scaber L.	Asteraceae	EA202H	Aerial part	19.79	13.86	12.08
		EA202C		3.24	3.07	1.69
		EA202B		>20	>20	>20
		EA202Aq		>20	>20	>20
Euphorbia prostata W. Ait.	Euphorbiaceae	EA213H	Whole plants	>20	>20	>20
		EA213C		>20	>20	>20
		EA213B		>20	>20	>20
		EA213Aq		>20	>20	>20
Excoecaria cochinchinensis Lour.	Euphorbiaceae	EA216H	Leaves	6.69	>20	>20
		EA216C		19.92	>20	>20
		EA216B		>20	>20	>20
		EA216Aq		>20	>20	>20
Justicia gendarussa Burm. F.	Acanthaceae	EA207H	Leaves	>20	>20	>20
		EA207C		>20	>20	>20
		EA207B		>20	>20	>20
		EA207Aq		>20	>20	>20
Kaempferia rotunda L.	Zingiberaceae	EA209H	Rhizome	>20	>20	>20
		EA209C		>20	>20	>20
		EA209B		>20	>20	>20
		EA209Aq		>20	>20	>20

Table 1. Continued

Plant name and Authority	Family	Smple code[a]	Part used	A549[b]	Col2[c]	SNU638[d]
Merremia mammosa (Lour.) Hallier F.	Convolvulaceae	EA211H	Tubera	>20	>20	>20
		EA211C		16.11	15.45	15.42
		EA211B		>20	>20	>20
		EA211Aq		>20	>20	>20
Parameria laevigata (Juss.) Moldenke	Apocynaceae	EA224H	Cortex	>20	>20	>20
		EA224C		>20	>20	>20
		EA224B		>20	>20	>20
		EA224Aq		>20	>20	>20
Ruellia tuberosa L.	Acanthaceae	EA222H	Aerial parts	>20	>20	>20
		EA222C		>20	>20	>20
		EA222B		>20	>20	>20
		EA222Aq		>20	>20	>20
Sindora sumatrana Miq.	Caesalpiniaceae	EA221H	Fructus	>20	>20	>20
		EA221C		>20	>20	>20
		EA221B		>20	>20	>20
		EA221Aq		>20	>20	>20
Strychnos ligustrina Bl.	Loganiaceae	EA208H	Lignum	>20	>20	>20
		EA208C		>20	>20	>20
		EA208B		>20	>20	>20
		EA208Aq		>20	>20	>20
Tinospora tuberculata Beumee	Menispermaceae	EA203H	Caulis	>20	>20	>20
		EA203C		>20	>20	>20
		EA203B		>20	>20	>20
		EA203Aq		>20	>20	>20
Vernonia cinerea (L.) Less.	Asteraceae	EA212H	Aerial parts	>20	>20	>20
		EA212C		>20	>20	>20
		EA212B		>20	>20	>20
		EA212Aq		>20	>20	>20
Zingiber cassumunar	Zingiberaceae	EA204H	Rhizome	>20	>20	>20
		EA204C		18.5	>20	11.31
		EA204B		>20	>20	>20
		EA204Aq		>20	>20	>20
Zingiber zerumbet (L.) J.E.Smith.	Zingiberaceae	EA198H	Rhizome	13.87	13.1	6.81
		EA198C		>20	>20	>20
		EA198B		>20	>20	>20
		EA198Aq		>20	>20	>20
Ellipticine				0.4	0.3	0.4

[a]Sample code : H (hexane), C (chloroform), B (butanol), Aq (aqueous).
[b]A549 : ED_{50} (μg/ml) in cultured Human lung cancer cells
[c]Col2 : ED_{50} (μg/ml) in cultured Human colon cancer cells
[d]SNU638 : ED_{50} (μg/ml) in cultured Human stomach cancer cells

lines. In addition, selective cytotoxic activity against test cancer cell lines was also found: *Excoecaria cochinchinensis* (A549 cells, ED_{50} = 6.69 μg/ml), *Eclipta alba* (Col2 cells, ED_{50} = 5.87 μg/ml), and *Azadirachta indica* (SNU638 cells, ED_{50} = 9.35 μg/ml).

In the course of our continuing screening of various plant extracts for potential cytotoxic antitumor compounds, the $CHCl_3$ extract of the rhizome of *Zingiber cassumunar* (Zingiberaceae) showed moderate cytotoxicity (ED_{50}<20 μg/ml) in A549 and SNU638 cancer cells. To the best of our knowledge, cytotoxic activity of *Z. cassumunar* has never been reported previously. Therefore, the fractionation of the $CHCl_3$ partition of the extract of *Z. cassumunar* was performed with silica gel column chromatography. Several subfractions were considered to be active as reported in the Table 2, and thus indicating that further bioassay-guided fractionations with these active fractions are encouraged for the discovery of new anticancer potentials. Novel anticancer agents might be expected to be isolated from these Indonesian plants by further study.

Table 2. Cytotoxic potential of subfractions of chloroform partition from *Z. cassumunar*

Fraction	A549[1)]	Col2[1)]	SNU638[1)]
F1	10.2	10.6	>20
F2	19.4	10.2	19.6
F3	>20	>20	>20
F4	19.3	12.9	17.3
F5	19.4	13.4	>20
F6	>20	11.9	>20
F7	>20	13.3	>20
F8	>20	15.2	>20
F9	>20	>20	>20
F10	>20	>20	>20
Ellipticine	0.3	0.8	0.4

[1)]Expressed by ED_{50} values in μg/ml.

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