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In-vitro antitumour evaluation of extracts from Cyanobacteria *anabaena*

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Pharmaceutical interest in algae and cyanobacteria (blue-green algae) as potential sources of biological active agents has increased in recent years. Cytotoxic or antitumour activity of crude extracts or purified compounds obtained from cyanobacteria have been reported (Gerwick et al 1989; Suzuki et al 1993). The present study is concerned with in-vitro cytotoxic activity of extracts from three strains of the freshwater cyanobacteria *Anabaena*, *A.cylindrica* M-1, *A.variabilis* M-3 and *A.variabilis* M-58, using mouse leukaemia L5178Y cells (RCB0135).

All cyanobacterial strains were grown photo-autotrophically in 500-mL cotton-wool-stoppered oblong flat flasks containing modified Detmer medium at 30°C and a light intensity of 2 klux for 7 d. The cultures were aerated by plain air. Dried and powdered cells from each cyanobacterial specimen were extracted sequentially with chloroform, ethanol and boiling water. Three kinds of dried extracts were then submitted to in-vitro cytotoxicity testing. L5178Y cells were incubated for 5d after addition of cyanobacterial extracts in a 96-well tissue culture microtitre plate. Initial dose levels at one-log interval (1 to 100 μ g mL^{-1}) with two tubes per level were tested based on the NCI protocol. Numbers of viable cells were counted in a haemocytometer. Cell viability was monitored by the Trypan blue exclusion method. Cytotoxic activity was evaluated as percentage of tumor cell growth compared with controls.

The chloroform extracts from three strains exhibited excellent cytotoxicity, while no activity was found in any of the aqueous extracts. All ethanol extracts exhibited moderate activity (Table 1).

Based on IC50 values (a measure of the 50% inhibitory concentration of test substance on tumour cell growth) determined in supplemental tests, the most promising chloroform extract from *A.variabilis* M-58 was fractionated by silica gel column chromatography using stepwise elution with hexane-chloroform-methanol, monitoring the fractions by the cytotoxic assay. The most active fraction was further separated and characterized by HPLC (ODS-W column; methanol-acetonitrile (25:75)) and spectral analysis.

Besides the synergistic effects of liphophilic model compounds found in cyanobacterial cells were examined since crude extracts contain many compounds and the combined use of cytotoxic drugs is effective in practice. A method was proposed to quantify the type and degree of interactions between two agents in the diagram.

Table 1. Cytotoxic activity (percent cell growth compared with controls) of extracts of cyanobacteria against mouse leukaemia L5178Y cells in-vitro.

Specimen	Extract	Dose (μ g mL^{-1})		
		100	10	1
M-1	Chloroform	32	88	99
	Ethanol	59	89	90
	Aqueous	104	100	93
M-3	Chloroform	27	87	97
	Ethanol	49	88	94
	Aqueous	100	93	89
M-58	Chloroform	30	87	96
	Ethanol	64	99	93
	Aqueous	104	91	92

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