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Mycoflora, Cytotoxicity, and DNA Interaction of Polyherbal Products from Malaysia

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Abstract

Eight polyherbal products sold in Malaysia were tested for mycoflora and the extracts analyzed for aflatoxins and ochratoxin A. Fungal count was low (less than 400 cfu/g) in all samples. *Aspergillus* spp. were isolated from all samples, but none of the isolates was mycotoxigenic. Other fungi isolated were *Eurotium* spp., *Cladosporium* spp., *Scopulariopsis* spp., *Phialophora* spp., *Fonseceae* spp., *Penicillium* spp. and *Paecilomyces* spp. Only one product was contaminated with mycotoxin: ochratoxin A. The herbal extracts were also tested for cytotoxicity on human cell lines Hep2 and HFL1 using the MTT assay. All extracts were cytotoxic to both cell lines at a concentration of 500 μg/ml. Four extracts were cytotoxic to both cells at 50 μg/ml but showed varying effects at 5 μg/ml. Five products, including those that were cytotoxic, interacted positively with DNA using the DNA–methylgreen assay. *In vitro* cytotoxicity tests showed that half of the products were cytotoxic and interacted with DNA.

Keywords: Aflatoxins, cytotoxicity, DNA interaction, herbal products, mycoflora, ochratoxin A, polyherbal.

Introduction

Herbal products are made from different parts of medicinal plant such as leaf, stem, root, flower, seed, or bark. The use of medicinal plants has increased in recent years in North America, Europe, Australia, and Southeast Asia (World Health Organization, 2005). In Malaysia, RM 2 billion (USD 0.5 billion) was spent on herbal medicine in 1997 (Hussein, 2001). There is a mythical yet predominant view that herbal medicines are harmless because they are "natural" (Bateman et al., 1998). However, Kaplowitz (1997) reported on health hazards associated with their use. The safety of several commercially available herbs has come into question due to reports of adverse reactions (Cupp, 1999). Therefore, some countries have implemented regulatory procedures on the safety and quality of herbal medicine (Bauer, 1998). The World Health Organization (WHO, 2004) classified an herbal drug with new combination of ingredients as "herbal medicine of uncertain safety" and also regards this product as a new substance. Safety data required for a new substance include toxicity testing. Most herbal products consumed in Malaysia are polyherbal, and there is very little or no information on the toxicity status of these products.

Herbal plant products may be contaminated with toxic substances, heavy metal, pesticides, or microorganisms during cultivation, harvesting, handling, and storage (Efuntoye, 1996; Zuin & Vilegas, 2000; Popat et al., 2001; Dwivedi & Dey, 2002; Kneifel et al., 2002). Tissue culture techniques have been used to evaluate the cytotoxicity of toxic plant extracts (Ruffa et al., 2002), heavy metals (Takano et al., 2002), and pesticides (Ivanov et al., 2001). Some investigators have also reported on the occurrence of toxigenic mycoflora and mycotoxin in herbal products (Aziz et al., 1998; Halt, 1998; Efuntoye, 1999) and their cytotoxic effects on animal and human cell lines (Kitabatake et al., 1993; Calvert et al., 2005). Tissue culture techniques using cultured human cells have been developed and have shown good correlation with data obtained using animal studies (Barile et al., 1995; Barile & Cardona, 1998).

Some herbal products have been found to be carcinogenic and mutagenic (Fu et al., 2002; Dasgupta, 2003). Evaluation of the binding activity of plant extract to DNA would provide useful information on the safety

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of herbal products. The objectives of this study were to determine the mycoflora and mycotoxin content of popular commercial herbal medical products marketed in Malaysia for fungal and mycotoxins content and to evaluate their cytotoxicity status *in vitro* using human cell lines and DNA binding activity.

Materials and Methods

Herbal products

Eight commercial herbal products were purchased from retail shops in Kuala Lumpur, Malaysia. The products were packaged in either bottle or plastic envelope, appropriately labeled, and were at least 6 months before the expiration date. Table 1 shows the formulation of the drugs and their intended uses.

Enumeration of fungi

Fungi were enumerated using the standard dilution method and direct plating on oxytetracycline–glucose–yeast extract agar (OGYE; CM545 Oxoid, Basingstoke, UK). In the dilution method, a 10 g herbal product was aseptically weighed into a sterile stomacher bag and 90 ml sterile Maximum Recovery Diluent was added. The mixture was stored at room temperature (20 ± 2°C) for 5 min and then homogenized in a stomacher (Steward Stomacher Blender 400, London, UK) for 1 min (Candlish et al., 2001). The homogenate was left for approximately 5 min to allow the coarse material to settle, and aliquots were plated onto the media. In direct plating method, the product (2 g) was aseptically sprinkled over the surface of the culture media with a sterile spatula. All plates were incubated at 25°C for 21 days. Fungal colonies were then subcultured on malt extract agar (MEA; CM59 Oxoid), incubated at 25°C for 72 h and identified using methods by Samson et al. (2002).

Preparation of herbal extracts

Extracts of the herbal drugs were used for the determination of mycotoxins, cytotoxicity assay, and DNA interacting activities. The extracts were prepared as described by Prozesky et al. (2001). Herbal product (20 g) was extracted with 150 ml methanol:chloroform mixture (1:1) for 24 h and filtered through Whatman no. 1 filter paper. For aflatoxin determination, the filtrate was evaporated in a rotary evaporator at 40°C to approximately 3 ml and then stored in dark glass vials at 2–4°C. For cytotoxicity assay and DNA interaction activities, the filtrate was first evaporated to dryness, weighed, and dissolved in dimethyl sulfoxide (DMSO) and ethanol, respectively.

Analysis of mycotoxins by thinlayer chromatography (TLC)

Aflatoxins (B_1, B_2, G_1, and G_2) and ochratoxin A (OTA) standards (Sigma-Aldrich, Poole, England) were dissolved in methanol and then diluted to 1 and 5 μg/μl, respectively. Herbal extract (20 μl) and mycotoxin standards were spotted onto precoated silica gel on aluminum sheet (20×20 cm), allowed to dry, and eluted in ethyl-acetate, toluene, and formic acid (7:5:5) solvent system (Samson et al., 2002). The plate was then examined under UV light at 365 nm. Mycotoxins in the sample were determined by comparing R_f values and fluorescence with the standards.

Maintenance of cell lines for cytotoxicity assay

Two human cell lines, Hep2 and HFL1 (purchased from the European Collection of Cell Culture, Salisbury, UK), were cultured as described by Whelan and Ryan (2003). The Hep2 cells were grown in a 75 cm^2 cell culture dish as a monolayer in minimum essential medium with Earles salts and glutamine (MEM; BioWhittaker, Berkshire, UK, BE12-611F) supplemented with nonessential amino acids (BioWhittaker Be1-114E), penicillin–streptomycin (100 IU/ml, Sigma P-0906), amphotericin B (0.25 μg/ml, BioWhittaker BE17-836E), and fetal bovine serum (10%, BioWhittaker). For HFL1, the cells were grown in a 75 cm^2 cell culture dish as a monolayer in Ham's F12 medium with glutamine supplemented with nonessential amino acids (BioWhittaker) and fetal bovine serum (10%, BioWhittaker). The culture dishes were incubated at 37°C in a CO_2 incubator (5% CO_2) for 72 h or until the cells showed 70–80% confluence.

Treatment of cell with herbal extracts

Treatment of the cell lines with the crude herbal extracts was carried out as described by Badisa et al. (2004). At approximately 80% confluence, the growth medium was removed by aspiration, and the cells were rinsed with 5 ml phosphate buffer solution (PBS). The washing was discarded, and 4 ml of trypsin (0.1%)/EDTA (0.04%) solution was added to the cells and incubated at 37°C for 5 min. After incubation, the cells were transferred to centrifuge tube with 6 ml phosphate buffer and centrifuged at 1000 rpm for 3 min. The supernatant was discarded, and 10 ml of appropriate growth medium (MEM or Ham's F12) was added. The cells were counted using a hemocytometer (Neubaur improved) and then diluted to approximately 5×10^5 cells/ml. Cell suspension (195 μl) was seeded onto a flat-bottom 96-well microtiter plate and incubated at 37°C for 24 h. The crude extracts were diluted in the appropriate growth medium in order to achieve required working stocks and added in a total volume of 5 μl per culture well. Each

Table 1. Herbal formulations and intended usage of some polyherbal preparations purchased from retail shops in Kuala Lumpur, Malaysia.

Herbal preparation	Constituents [Species (family)]	Plant parts used[a]	Percentage in the formulation (%)	Intended use
Pasak Bumi (PB)	*Eurycoma longifolia* Jack. (Simaroubaceae)	Radix	40	Increase passion in women.
	Curcuma longa L. (Zingiberaceae)	Rhizoma	10	
Maajun Ratu (MR)	*Zingiberis minus* Gaertn. (Scitamineae)	Rhizoma	5.6	For smooth menstrual flow, and to relieve dysmenorrhea, to relieve joints pain.
	Eugenia caryophyllata Thunb. (Myrtaceae)	Flos	11.1	
	Piper nigrum L. (Piperaceae)	Fructus	11.1	
	Illicium verum Hook. f. (Illiciaceae)	Flos	11.1	
	Carum copticum Benth. & Hook.f. (Umbelliferae)	Semen	16.7	
	Astragalus membranaceus Bunge (Leguminosae)	Radix	22.2	
	Angelicae sinensis (Oliv.) Diels. (Umbelliferae)	Rhizoma	21.3	
Tongkat Ali (TA)	*Eurycoma longifolia* Jack. (Simaroubaceae)	Radix	50	To increase sexual stamina and energy in men.
	Cistanche deserticola Y.C.Ma. (Scrophulariaceae)	Herba	50	
Greennleaf Energizer (GE)	*Eurycoma longifolia* Jack. (Simaroubaceae)	N.I.	30.4	For energy, increase sexual stamina, and men's health.
	Tacca integrifolia Ker Gawl. (Taccaceae)	N.I.	21.4	
	Curcuma aromatica Salisb. (Zingiberaceae)	N.I.	17.9	
	Zingiber officinale Roscoe (Zingiberaceae)	N.I.	14.3	
	Helminthostachys zeylanica (L.) Hk. (Pteridophytae)	N.I.	16	
Medicare AM700 (MAM700)	*Verbena officinalis* L. (Verbenaceae)	Herba	12.5	Promote blood circulation, relieve minor edema, and relieve pain.
	Sparganium stoloniferum Buch.-Ham (Typhaceae)	Rhizoma	12.5	
	Curcuma longa L. (Zingiberaceae)	Rhizoma	12.5	
	Hedyotis diffusa Spreng. (Rubiaceae)	Herba	17	
	Selaginella doederieini Hieron. (Pteridophytae)	Herba	17	
	Scutellaria barbata D. Don (Labiatae)	Herba	12.5	
	Astragalus membranaceus Bunge (Leguminosae)	Radix	8	
	Rehmannia glutinosa Libosch. (Scrophulariaceae)	Radix	8	
Medicare AM800 (MAM800)	*Astragalus membranaceus* Bunge (Leguminosae)	Radix	10	Promote blood circulation, to clear phlegm, relieving cough and pain.
	Cinnamomum cassia Blume. (Lauraceae)	Ramulus	10	
	Ephedra sinica Stapf. (Ephedraceae)	Herba	10	
	Prunus armeniaca Thunb. (Rosaceae)	Semen	10	
	Schisandra chinensis (Turcz.) Baill. (Schisandraceae)	Fructus	10	
	Perilla frutescens (L.) Britton (Labiatae)	Fructus	10	
	Lepidium apetalum Willd (Brassicaceae)	Semen	10	
	Trichosanthes rosthornii Harms. (Cucurbitaceae)	Fructus	10	
	Aster tataricus Turcz. (Compositae)	Radix	10	
	Glycyrrhiza uralensi L. (Fabaceae)	Radix	10	

(*Continued*)

Table 1. Continued.

Herbal preparation	Constituents [Species (family)]	Plant parts used[a]	Percentage in the formulation (%)	Intended use
Qu Ban Ling (QBL)	*Spina gleditsiae* Lam. (Fabaceae)	Radix	22.3	For general health.
	Angelicae sinensis (Oliv.) Diels (Umbelliferae)	Radix	16.7	
	Salvia miltiorhizae Bunge (Labiatae)	Fructus	16.7	
	Zizyphus sativa Gaertn. (Rhamneae)	N.I.	4.5	
	Cordycep sinensis (Berk) Sacc. (Clavicipiteae)	Cordex	11	
	Dictamnus dasycarpus Turcz. (Rutaceae)	Radix	6.8	
	Rhodiola sacra (Raymond Hamet) Fu (Crassulaceae)	Herba	11	
	Schizonepeta tenuifolia Briq. (Labiatae)	Herba	11	
Ming Muh Dih (MMD)	*Rehmannia glutinosa* Steud. (Scrophulariaceae)	Radix	21.4	For general health.
	Paeonia suffruticosa Andrews (Paeoniaceae)	Cordex	7.2	
	Paeonia emodi Royle (Paeoniaceae)	Radix	7.2	
	Alisma plantago-aquatica L.(Alismataceae)	Rhizoma	7.2	
	Chrysanthemum morifolium Ramat. (Compositae)	Flos	7.2	
	Lycium barbarum Lam. (Solanaceae)	Fructus	7.2	
	Tribulus terestris L. (Zygophyllaceae)	Fructus	7.2	
	Dioscorea opposite Thunb. (Dioscoreaceae)	Rhizoma	9.4	
	Cornus officinalis Siebold & Zucc.(Cornaceae)	Fructus	9.4	
	Angelica sinensis (Oliv.) Diels. (Umbelliferae)	Radix	9.4	

[a]Plant parts used: N.I., not indicated; Radix, the root; Rhizoma, rhizome or a creeping horizontal stem generally bearing roots on its underside; Flos, the flowers; Fructus, the fruit or berry; Semen, the seed usually removed from the fruit and may or may not contain the seed coat; Herba, the aerial parts or the aboveground parts of plants, which may include the flower, leaf, and the stem; Cordex, the bark collected from the root, stem, or branches; Ramulus, a small branch.

concentration was done in quadruplicate, and three different concentrations (500, 50, and 5 μg/ml) were evaluated. Untreated cell lines served as control. All culture plates were incubated at 37°C in a CO_2 incubator (5% CO_2) for 72 h.

Cytotoxicity test of herbal extracts

Cytotoxicity of the herbal extracts was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method based on the respiratory activity of the mitochondrial succinate–tetrazolium reductase system, which converts the yellow tetrazolium salt to a blue formazan dye. The amount of formazan produced is proportional to the amount of living cells in the culture. The procedure described by Betancur-Galvis et al. (1999) was employed in this study. After incubation of the cell lines with the extracts, the supernatants were removed and 25 μl of MTT in PBS (2 mg/ml) was added to each well, and the plates were incubated again in a CO_2 incubator (5% CO_2) at 37°C for 2 h. Then, 125 μl dimethyl sulfoxide (DMSO) was added to dissolve any intracellular formazan crystals and agitated for 15 min on a rotary shaker. Absorbance was measured in an ELISA plate reader (Dynex MRII, Worthing, UK) at 492 nm. Absorbance of untreated cells served as control and background absorbance from wells containing no cells as a blank. The percentage of cytotoxicity was calculated using the formula,

$$[(A - B)/A]/ \times 100$$

where A is the mean optical density of control wells, and B is the optical density of wells with plant extracts.

Assay for DNA-interacting activity

This assay is based on binding of a dye to DNA to form a reversible DNA/methyl green complex that is then used as substrate to measure DNA-binding activity. When the dye is displaced from DNA, water molecule then reacts with methylgreen to form a colorless molecule: carbinol. The reaction can then be followed spectrophotometrically due to decrease in absorbance. Assay for DNA-interacting activity was done using the method described by Mongelli et al. (2000) with slight modifications. Herbal extracts (20 μl) in methanol was dispensed in 96-well microplate (6 for each sample) and the solvent was evaporated under vacuum. A DNA–methylgreen reagent was prepared by dissolving 20 mg of DNA-MG (Sigma, Poole, Dorset, UK) in 100 ml of 0.05 M Tris-HCl buffer, pH 7.5, containing 7.5 mM $MgSO_4$ and stirred at 37°C for 24 h. Two-hundred microliters of the reagent (200 μl) was added to each well, and initial absorbance of each sample was read at 655 nm using a microplate reader (Dynex MRII,

Table 2. The examination of the herbal preparation for total viable fungal content and the presence of aflatoxins and ochratoxin A.

Herbal preparation	Fungal viable count[a] (log_{10} cfu/g)		Fungi isolated	Mycotoxins detected
	Direct culture	Dilution method		
PB	<1.0	<1.0	*Aspergillus* spp. *Eurotium* spp.	–
MR	<1.0–1.5	<1.0–1.1	*Aspergillus* spp. *Eurotium* spp. *Paecilomyces* spp.	–
TA	<1.0	<1.0	*Aspergillus* spp.	–
GE	<1.0–1.3	<1.0	*Aspergillus* spp.	–
MAM700	<1.0–1.7	<1.0	*Aspergillus niger* *Aspergillus* spp. *Cladosporium* spp. *Scopulariopsis* spp. *Phialophora* spp. *Fonsecaea* spp. *Eurotium* spp.	–
MAM800	1.1–1.5	<1.0–2.4	*Aspergillus* spp. *Eurotium* spp.	Ochratoxin A
QBL	2.1–2.4	2.4–2.6	*Aspergillus niger* *Aspergillus* spp. *Penicillium* spp.	–
MMD	<1.0–1.1	<1.0	*Aspergillus niger* *Paecilomyces* spp.	–

cfu/g, colony-forming unit per gram sample; –, not detected. See Table 1 for full names of herbal preparations.
[a]Values were averaged of counts on four different culture media.

Worthing, UK). The plate was incubated in the dark at room temperature ($20 \pm 2^{\circ}C$) for 18 h and absorbance read. Doxorubicin hydrochloride (10 mg/ml in water) was used as control.

Results

Table 2 shows the fungal counts, fungal isolates, and mycotoxin present in the polyherbal products tested. Fungal counts for both direct plating and dilution techniques were similar. Samples MAM800 and QBL showed fungal counts of $\log_{10}$ 2.4 and 2.6, respectively, whereas the counts in samples GE, MAM700, and MMD were less than 1.7 cfu per gram. Samples TA and MMD showed little or no counts. *Aspergillus* spp. were isolated from all samples but mycotoxigenic species such as *A. flavus, A. parasiticus, A. ochraceous, and A. carbonareus* were not present. Other fungi isolated were *Eurotium* spp. (50% of samples), *Paecilomyces* spp. (25%), *Cladosporium* spp. (13%), *Scopulariopsis* spp. (13%), *Phialophora* spp. (13%), *Fonseceae* spp. (13%), and *Penicillium* spp. (13%). Of the mycotoxins tested, only one sample (MAM800) contained OTA. Table 3 shows the cytotoxic activity of the herbal extracts at three different concentrations on HFL1 and Hep2 cells. All extracts exhibited at least 50% cytotoxicity on both cell lines at 500 μg/ml. However, four samples (PB, MR, TA, and GE) exhibited at least 50% toxicity at 50 μg/ml on both cell lines. At concentration of 5 μg/ml, samples MR, TA and GE showed at least 50% cytotoxic activity on HFL1 cell lines while samples TA and GE exhibited 25 to 50% toxicity on Hep2 cell lines. Extracts of samples MAM700, MAM800, QBL, and MMD showed little or no cytotoxic effects on both cell lines at 5 and 50 μg/ml. Table 4 shows interaction of herbal extracts with DNA. Absorbance decrease of 20% or more was considered a significant activity and therefore signified positive reaction. Extracts from samples PB and MAM800 caused decrease in absorbance of 41% and 32%, respectively, whereas samples MR, TA, and GE showed 24% decrease. The remaining samples showed 11% to 19% decrease.

Discussion

Enumeration of fungi in the herbal products showed that the counts in these drugs were lower than the limit of fungal count set by some countries. In Malaysia, fungal count in herbal products should be less than 2.6 cfu per gram (National Pharmaceutical Control Bureau of Malaysia, 1999). Hitokoto et al. (1978) examined 49 powdered herbal samples and reported that 45 samples contained less than 2.6 cfu per gram and none contained mycotoxins. They noted that the low fungal count was due to good manufacturing practice, low water content of the products because of the prevelance of *Eurotium* spp. in the samples, and the use of airtight packaging. In this study, *Eurotium* spp. were present in 50% of the samples tested. *Aspergillus* spp. were isolated in all herbal samples, but toxigenic species were not present, although previous studies reported the presence of aflatoxigenic fungi (Hitokoto et al., 1978; Tassaneeyakul et al., 2004).

Analysis by TLC showed that all samples were free of mycotoxins except sample MAM800, which was positive for OTA. Tassaneeyakul et al. (2004) examined 28 herbal medicinal products in Thailand using high-performance liquid chromatographic (HPLC) technique and found that 5 samples were contaminated with aflatoxins ranging from 1.7 to 14.3 ng/g, all below the current legislative level (20 ng/g) permissible in Thailand. These findings suggest that commercial herbal products may be contaminated with mycotoxins but at very low level. The presence of OTA in MAM800 indicates the toxin was preformed in the sample. Moreover, Kitabatake et al. (1993) and Lewis et al. (1999) reported that concentrations of some mycotoxins below the detection limit by TLC did not affect many cell lines.

Cytotoxicity tests of the extracts on HFL1 (normal cells from human embryonic lung) and Hep2 (cells from

Table 3. Cytotoxicity of herbal extracts (5, 50, and 500 μg/ml) on two cell lines (HFL1 and Hep2).

	HFL1 cells			Hep2 cells		
Herbal preparation	500	50	5	500	50	5
PB	+ + +	+ +	–	+ + +	+ +	–
MR	+ + +	+ + +	+ +	+ + +	+ +	–
TA	+ + +	+ + +	+ +	+ + +	+ + +	+
GE	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
MAM700	+ +	+	–	+ +	–	–
MAM800	+ +	–	–	+ +	–	–
QBL	+ +	–	–	+ +	–	–
MMD	+ +	–	–	+ +	–	–

–, indicates 100–75%; +, 75–50%; + +, 50–25%; + + +, 25–0% cell proliferation against control (wells containing nontreated cells). See Table 1 for full names of herbal preparations.

Table 4. Interaction of herbal extracts with DNA–methyl green (n = 6).

Polyherbal remedies	% of absorbance decrease (mean ± SD)	DNA interaction
Doxorubicin	65.43 ± 0.70	Positive (control)
PB	41.2 ± 2.3	Positive
MR	24.3 ± 4.3	Positive
TA	24.1 ± 0.5	Positive
GE	24.4 ± 0.6	Positive
MAM700	18.9 ± 0.1	Negative
MAM800	32.2 ± 1.8	Positive
QBL	16.1 ± 0.3	Negative
MMD	10.7 ± 0.7	Negative

See Table 1 for full names of herbal preparations.

laryngeal carcinoma) indicated that samples GE and TA were the most cytotoxic while PB and MR were moderately cytotoxic. Sample GE inhibited 75–100% and 50–75% HFL1 and Hep2 cell growth, respectively, at very low concentration of 5 μg/ml, and TA inhibited 50% to 75% growth of HFL1 at 5 μg/ml. Samples GE, TA, and PB contained *Eurycoma longifolia* Jack. that has been reported to be cytotoxic (Jiwajinda et al., 2002). However, there is no known or reported adverse effects of *E. longifolia*, which is consumed orally. *Eurycoma longofolia* extract was found to be 100-times less cytotoxic if administered orally rather than intraperitoneally in experimental mice (Satyavivad et al., 1998). In TA, *E. longifolia* was mixed with one constituent, *Cistanche deserticola* Y.C.Ma., while in GE it was mixed with four [*Tacca integrifolia* Ker Gawl, *Curcuma aromatica* Salisb., *Zingiberis officinale* Roscoe, and *Helminthostachys zeylanica* (L.) Hk.]. To date, there seems to be little or no information on cytotoxicity of herbal constituents *Tacca integrifolia, Curcuma aromatica, Zingiberis officinale*, and *Helminthostachys zeylanica*. Thus, higher cytotoxicity of GE as compared with TA could be due to interaction between the chemical constituents of two or more herbal extracts in GE synergistically resulting in higher cytotoxicity effects. In contrast, MAM700, QBL, and MMD contain 8 to 11 plant ingredients but were not cytotoxic. In these products, many constituents of the extracts may have antagonistic effects (Chung et al., 2004). Jestoi et al. (2004) studied the levels of mycotoxins and sample cytotoxicity of commercial grain-based products and noted that although all samples evoked cytotoxicity for feline fetal lung cell line (FL), there was no correlation with mycotoxins, which were found to be low in all samples. They concluded that FL cells could be used for the measurement of cytotoxicity if the concentrations of mycotoxins were high and that the cytotoxic effects exhibited by the samples were attributable to compounds other than mycotoxins. Calvert et al. (2005) reported that mycotoxins from *Fusarium* spp. exhibited time- and concentration-dependent cytotoxicity on some human cell lines. Results from this study indicate that the high cytotoxicity activities of GE and TA were mainly attributed to substances other than mycotoxins in these herbal remedies. This is in agreement with the findings of Jestoi et al. (2004). This study, therefore, indicates that cytotoxicity testing is appropriate and essential for herbal products. In Malaysia, toxicity testing is not compulsory for traditional herbal products as long as the formulation contains plant constituents that have been used in traditional medicine (National Pharmaceutical Control Bureau of Malaysia, 2001).

The results showed that sample PB gave the highest decrease (41%) in absorbance and, therefore, it was more reactive with DNA than the remaining samples, and this finding is significant in view of the fact that doxorubicin, a substance that interacts strongly with DNA, showed a 66% absorbance decrease at 10,000 μg/ml. The interaction with DNA can be exerted in different ways such as intercalative binding, non-intercalative binding, covalent binding and binding followed by DNA-strand scission (Bonjean et al., 1996). The nucleic acid assay is important for two reasons: product that interacts positively with DNA indicates its mutagenic potential but, on the other hand, it also shows its potential as cancer chemotherapeutic agent (Pezzuto et al., 1991). Results from this study indicate that herbal medicinal products (except MAM800) that positively reacted with DNA were cytotoxic as well on Hep2. However, there was no correlation between the degree of cytotoxicity and DNA interaction. This suggests that interaction of the herbal drugs with cellular DNA may lead or partially lead to cell cytotoxicity. Because this test is relatively simple and rapid to perform, it could be considered as part of testing batteries for herbal products.

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