Natural Product Sciences
1(1) : 31 ~ 42 (1995)

Tumour Promoting Activity of Plants Used in Malaysian Traditional Medicine

M. Ilham[1], M. Yadav[2] and A.W. Norhanom[3]

Institute of Advanced Studies; [2]Department of Genetics and Cellular Biology; and
[3]Centre for Foundation Studies in Science, University of Malaya, 59100 Kuala Lumpur, Malaysia

Abstract—One hundred plants in 43 families used in Malaysian traditional medicine were screened for tumour promoting activity using two methods, the technique of activation of latent Epstein-Barr Virus (EBV) in Raji cells and the irritation test on mouse ear. Extracts of only eight plants belonging to the Euphorbiaceae were found to possess EBV activation factor and to give a positive irritation test in mouse ear. These plants included *Euphorbia tirucalli* L., *E. splendes*, *Jatropha podagrica*, *J. gossypyfolia* L., *Pedilanthus tithymaloides* (L.) Poitt., *Croton argyratus* Bl., *Exocoecaria agallocha* L. and *Codiaeum variegatum* (L.) Bl. Seven of these plants are used internally in Malaysian traditional medicine. As such, they pose potential danger in the promotion of initiated cells of the mucosal tissue towards disease. Further studies are required to assess the epidemiological impact of these plants in the development of disease.

Keywords—medicinal plants tumour promoter activity

About 1,300 species of Malaysian plants are recognised to have medicinal properties (Burkill, 1966). However, systematic laboratory investigations on the safety and efficacy of these plants against specific ailments are lacking.

Plants are known to yield powerful tumour promoters which may cause secondary effects if regularly ingested (Evans, 1986). In fact several epudemiological studies reveal circumstantial associations between plants containing tumour promoters and the increased incidence of human cancers (Hecker, 1987; Ito, *et al.*, 1986).

Zur Hausen *et al.* (1978, 1979) demonstrated that phorbol esters which possessed tumour promoting activity could induce viral cycle in latently infected cells carrying Epstein-Barr virus (EBV), whereas chemically related compounds lacking tumour promoting activity did not. Ito *et al.* (1981) noted that low concentration of n-butyrate increased the effects of tumour promoters synergistically, while naturally occurring tumour promoters have powerful irritant effect on mouse skin (Fujiki *et al.*, 1979). In the present study we have used the butyrate synergistic assay and the irritant test on mouse ear (Fujiki *et al.*, 1979) as a rapid method for screening selected plants.

Since in an earlier study (Yadav *et al.*, 1989) we noted that a high proportion of plants from the family Euphorbiaceae used in traditional medicine possessed EBV-inducing activity, in the present study we screened and evaluated common plants used in Malaysian traditional medicine for their potential tumour promoting activity. Such information was considered important in view of the finding that tumour promoters in herbal preparations may act as cofactors in the induction of tumours in persons who used the herbs regularly as medication (Ito *et al.*, 1986). Moreover, such a study

[3]Author for correspondence.

has never been performed previously for Malaysian medicinal plants.

Experimental

Plants were collected from the University of Malaya Botanical Garden (Rimba Ilmu) and the botanical names were authenticated.

Leaves, stem or seeds of the plants were air dried and then ground in a mill to a fine powder before extraction. About 5 gm of the ground plant was extracted in 10 ml of ether at room temperature for 24 h. The extract was removed form the plant debris by centrifugation and vacuum- dried in a Savant Speedvac concentrator. The dry crude extract was dissolved in dimethylsulfoxide (DMSO) to provide a stock solution of 10 mg/ml. The solution was sterilized using a 0.45 μm filter membrane and stored at -20°C until used.

Assay for induction of Epstein-Barr Virus early antigen - Raji cells were obtained from the National Cancer Institute, Bethesda, Maryland (U.S.A.) (courtesy of Dr. D.V. Ablashi). The culture medium consisted of RPMI 1640 medium supplemented with L-glutamine (0.2 g/L), inactivated fetal calf serum (10%), streptomycin (100 μg/ml) and penicillin (100 IU/ml). Cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Rapidly dividing cells at density of 1×10^6 cells/ml were incubated with the plant extract at various concentrations in the presence of 4 mM sodium n-butyrate for 72 hours in a humidified incubator at 37°C with 5% CO_2 in air. The positive control consisted of Raji cells treated with optimal concentration of 12-0-tetradecanoyl-13-acetate (TPA), while the negative controls consisted of untreated cells. In previous studies it had been established that optimal early antigen activity was obtained at 72 h (Yadav *et al.*, 1989).

The Raji cells were harvested on 3-day by centrifugation at 1,000 rpm for 5 min and washed 3X in phosphate buffer saline (PBS). The cells were then resuspended in PBS and 50 μl were dispensed onto wells of teflon-coated slides. The evenly spread cell smears were dried in cold air, fixed in cold acetone at -20°C for 10 min and used in indirect immunofluorescence assay as previously described (Norhanom *et al.*, 1987). Briefly, the fixed Raji cells were overlaid with 20 μl of serum containing IgG antibody to EBV-EA or control sera negative for EBV anti-EA antibody. The slides were incubated in a humidified chamber at 37°C for 45 min after which they were rinsed with PBS for 5 min. Such slides were further overlaid with 20 μl of anti-human IgG conjugated to fluorescein isothiocynate and incubated for 45 min at 37°C and again rinsed with PBS.

When dry, the slides were mounted in glycerol-PBS (9:1) buffer and the number of positive cells counted under the microscope equipped with epi-ultraviolet source, BG 12 exciter filter, BG 38 suppressor filter and 530 barrier ocular filter. Cells with intensely brilliant intracellular fluorescence were classified as positive, while cells with dull, diffuse or no fluorescence were scored as negative. The proportion of positive cells were recorded as a percentage of total cells counted.

Assay for irritant activity on Mouse Ear - Eight weeks old, female ICR mice were obtained from University of Malaya's Animal House. The mice were housed in stainless steel cages in groups of 4 per cage and fed with commercial basal diet pellet and water ad libitum.

An aliquot of 10 μl of the extract in acetone at the concentration of 10 mg/ml was applied on the inner surface of the right outer ear of each mice. Eight solutions of two-fold serial dilution were prepared for each plant extract and similarly tested. The left ear was left untreated and was used as a reference in order to evaluate the degree of ear redness. Mouse ears were examined macroscopically 24 h after administration of the test compound. The

degree of redness was numerically scored as 0 (normal appearance), 1 (slight reddening of the areas in between, 25% irritant), 2 (marked reddening of the main vessels with mild reddening of the areas in between, 50% irritant), 3 (intense reddening of the entire ear often combined with macroscopically visible hyperplasia, 75% irritant) or 4 (cell damage-necrosis, 100% irritant). The reddening of the ear was reversible.

Results

Induction of EBV antigens by plant extracts - Ninety-eight extracts of 98 species corresponding to 43 different families were investigated. Table 1 provides the plant's name, part tested, medicinal uses, EBV-EA induction, and the irritant activity of the extracts on mouse ear. Among the 98 plants tested at concentrations of 500 and 1,000 ng/ml, eight species belonging to the Euphorbiaceae (*Euphorbia tirucalli, E. splendes, Jatropha podagrica, J. gossypyfolia, Pedilanthus tithymaloides, Croton argyratus, Excoecaria agallocha* and *Codiaeum variegatum*) showed the presence of EA-inducing activity and caused irritation on mice ear. The negative control Raji cells incubated with 4 mM n-butyrate showed the maximum EA induction of 1% as

Table 1. Demonstration of Epstein-Barr Virus early antigen and mouse ear irritant activity of extract of plants used in Malaysian traditional medicine.

Family/botanical name	Plant parts[a]	Uses(s) property[b]	EA induction[c]	Skin irritation[d]
ACANTHACEAE				
Andrographis paniculata Nees	LF	analgesic, a poultice	0	0
Graptophyllum picium (L.) Griffith	LF	emolient, resolvent, a poultice	0	0
Justicia gendarusa Burm.	LF	diuretic, laxative, analgesic, diaphoretic, a poultice	0	0
AMARANTHACEAE				
Gomphrena globosa L.	LF	a poultice	0	0
ANNONACEAE				
Annona muricata L.	LF	astringent, maturative, a poultice, styptic (external)	0	+
APOCYNACEAE				
Nerium oleander L.	LF	diuretic restorative, tonic (cardiotonic)	0	0
Plumeria acutifolia Poir.	LF	emmenagogue, febrifuge, purgative, diuretic	0	0
Rauvolfia serpentina Benth.	LF	purgative	0	0
Catharanthus roseus (L) G. Dont	LF	astringent diaphoretic, expectorant, emmenagogue	0	0
ARACEAE				
Dieffenbachia reginae L.	LF	a poison, antirheumatic	0	0
Rhaphidophora minor Hook.	LF	stimulant, aphrodisiac, antihelmintic	0	0

Table 1. Continued

Family/botanical name	Plant[a] parts	Uses(s)[b] property	EA[c] induction	Skin[d] irritation
ARALIACEAE				
Nothopana scutellaria (Burm.) Merr.	LF	diuretic, sudorific	0	0
Polyscias fruticosa (L.) Harms	LF	diuretic, sudorific	0	0
BIGNONIACEAE				
Crescentia cujette L.	LF	demulcent, emollient, expectorant, antitussive	0	0
CAPRIFOLIACEAE				
Sambucus javanica Reinw. ex Bl.	LF	antirheumatic, analgesic	0	0
COMPOSITAE				
Blumea balsamifera (L.) DC.	LF	stimulant sudorific	0	0
Emilia sonchifolia (L.) DC.	LF	bechic, antitussive, tonic (ophthalmic)	0	0
Eupatorium odoratum L.	LF	digestive, stimulant, analgesic	0	0
Wedelia biflora DC.	LF	diuretic, laxative, tonic (prophylatic)	0	0
CRASSULACEAE				
Bryophyllun calycinum Salisb.	LF	antibiotic, expectorant, antitussive	0	0
CUPRESSACEAE				
Cupressus semperivrens L.	LF	purgative	0	0
EUPHORBIACEAE				
Acalypha hispida Burm. f.	LF	maturative	0	0
A. indica L.	LF	expectorant, antitussive, purgative	0	0
A. siamensis Oliv. ex Gage	LF	diuretic, a poultice	0	0
A. wilkesiana Moorea	LF	diuretic	0	0
A. wilkesiana Macafeana	LF	diuretic	0	0
Baccaurea dulcis Muell. Arg.	BK	vermifuge stomachic	0	0
Cicca accidia Merr.	BK	emetic, laxative, a poultice	0	0
Codiaeum variegatum (L.) Bl.	LF	contraceptive purgative, a poultice	+	+
Croton argyratus Bl.	LF	purgative, post-partum treatment	+	+

Table 1. Continued

Family/botanical name	Plant[a] parts	Uses(s)[b] property	EA[c] induction	Skin[d] irritation
Euphorbia hirta L.	LF	a poultice, expectorant	0	0
E. tirucalli L.	ST	emetic, a poultice, purgative	+	+
E. pulcherrima Willd.	ST	a poultice purgative	0	0
E. splendes	ST	purgative	+	+
Exocoecaria agallocha L.	ST	purgative	+	+
Homalanthus populneus L.	LF	analgesic, antirheumatic	0	0
Jatropha gossypyfolia L.	LF	emetic, purgative	+	+
J. podagrica Hook.	SD	emetic, purgative	+	+
Phyllanthus frondosus Wall. ex Muell-Arg.	LF	purgative	0	0
P. niruri L.	LF	expectorant, emmenagogue	0	0
P. reticulatus Poir.	LF	astringent, diuretic	0	0
Pedilanthus tithymaloides (L.) Poitt.	ST	antidote, vulnerary	+	+
GUTTIFERAE/CLUSIACEAE				
Garciia cowa Roxb.	LF	tonic	0	0
ILLICIACEAE				
Illicium cyminum Boldingh	LF	carminative stimulant antirheumatic	0	0
Illicium verum Hook.	LF	carminative,	0	0
LABIATAE				
Orthosiphon aristatus (Bl.) Miq.	TW	diuretic	0	0
Coleus blumei Benth.	LF	purgative	0	0
Leucas zeylanica R. Br.	LF	a poultice, vermifuge	0	0
LAMIACEAE				
Ocimum basilicum L.	SD	demulcent, stimulant, diuretic	0	0
LAURACEAE				
Cinnamomum cassia Bl.	BK	febrifuge, stimulant	0	0
LEGUMINOSAE				
Parkia roxburghii G. Don	SD	carminative, a poultice	0	0
Cassia occidentalis L.	BK	analgesic, antiherpetic, vulnerary	0	0
Cassia alata L.	LF	laxative, purgative antihelmintic	0	0
Tamarindus indica L.	LF	expectorant	0	0
LILIACEAE				
Allium ascalonicum L.	BU	alternative, resolvent, vulnerary	0	0

Table 1. Continued

Family/botanical name	Plant[a] parts	Uses(s)[b] property	EA[c] induction	Skin[d] irritation
A. sativum L.	BU	carminative, antiseptic, expectorant	0	0
Aloe vera L.	LF	laxative, a lotion, purgative	0	0
Cordyline terminalis (L.) Kunth	LF	analgesic, vulnerary	0	0
Dracaena graminifolia Wall.	LF	galactogogue, hair restorative	0	0
D. surculosa paniculata	LF	galactogogue	0	0
Sanseviera trifasciata Prain	LF	antidote, a poultice	0	0
LYTHRACEAE				
Lawsonia inermis L.	LF	a poultide, diuretic, vulnerary	0	0
MALVACEAE				
Hibiscus rosa-sinensis L.	LF	a poultice, emollient, expectorant	0	0
MARANTACEAE				
Marantha arundinacea L.	LF	a poultice	0	0
MELASTOMACEAE				
Melastoma malabathricum L.	LF	astringent, hematic	0	0
MELIACEAE				
Azadirachta indica A. Juss.	LF	analgesic, antipyretic	0	0
MENISPERMACEAE				
Tinospora crispa (L.) Miers	ST	emetic, febrifuge, stomachic	0	0
MYRISTICACEAE				
Myristica fragranse Houtt.	SD	carminative, stomachic, astringent	0	0
MARTACEAE				
Psidium gujava L.	LF	stomachic. astringent, vermifuge	0	0
NYGTAGINACEAE				
Mirabilis jalapa L.	LF	purgative	0	0
PALMAE				
Areca catechu L.	LF	digestive, astringent, emmenagogue	0	0
Cocos nucifera L.	FM	laxative, antidiarrhoeic, diuretic	0	0

Table 1. Continued

Family/botanical name	Plant[a] parts	Uses(s)[b] property	EA[c] induction	Skin[d] irritation
PANDANACEAE				
Pandanus odorus Ridl.	LF	post partum treatment	0	0
PIPERACEAE				
Piper betle L.	LF	carminative, stimulant, expectorant	0	0
P. nigrum L.	SD	stimulant, rubefacient, poultice	0	0
P. sarmentosum Roxb.	LF	analgesic	0	0
PLUMBAGINACEAE				
Plumbago sp.	LF	vesicant	0	0
PUNICACEAE				
Punica granatum L.	LF	tonic	0	0
PORTULACACEAE				
Talinum sp.	LF	tonic	0	0
ROSACEAE				
Eriobotrya japonica (Thunb.) Lindl.	LF	stomachic	0	0
RUBIACEAE				
Uncaria sp.	LF.	poultice, astringent	0	0
RUTACEAE				
Murraya paniculata (L.) Jack	LF	astringent, antidysenteric, febrifuge	0	0
Aegle marmelos Correa	LF	analgesic, abortifacient	0	0
SIMAROUBACEAE				
Eurycoma longifolia Jack	BK	febrifuge, or poultice, aphrodsiac	0	0
SOLANACEAE				
Datura metel L.	SD	sedative, analgesic	0	0
Capsicum annuum L.	FR	stomachic, rubefacient	0	0
Nicotiana tabacum L.	LF	sedative, a poultice	0	0
UMBELLIFERAE				
Coriandrum sativum L.	SD	analgesic, post partum treatment	0	0
Cuminum cyaminum L.	LF	antiemetic a lotion	0	0
URTICACEAE				
Artocarpus integer (Thunb.) Merr.	FR	a poultice, antisyphilitic, vermifuge	0	0

Table 1. Continued

Family/botanical name	Plant[a] parts	Uses(s)[b] property	EA[c] induction	Skin[d] irritation
VERBENACEAE				
Clerodenderun fragrans (Vent.) Willd.	LF	febrifuge, a poultice	0	0
Stachytarpheta indica (L.) Vahl	LF	purgative, antihelmintic	0	0
ZINGIBERACEAE				
Alpinia galanga (L.) Willd.	LF	a poultice, post partum treatment	0	0
Costus speciosus (Koenig.) Smith	LF	sudorific, a poultice	0	0
Curcuma domestica Val.	RH	stimulant, carminative, hematic	0	0
Curcuma xanthorrhiza Roxb.	LF	laxative, tonic (hepatic, renal)	0	0
Gastrochillus panduratus (Roxb.) Ridl.	RH	stomachic	0	0
Kaempfera galanga L.	LF	antitussive, a poultice, expectorant, carminative	0	0

[a]BK: Bark; BU: Bulb; FR: Fruits; FL: Flower; LF: Leaves; RH: Rhizomes; ST: Stem; FM: Fruit milk; SD: Seed; TW: Twigs.
[b]Burkill, 1966; Perry and Metzger, 1980.
[c]+: positive for EBV EA induction
0: negative for EBV EA induction
[d]+: positive for skin irritation assay
0: negative for skin irritation assay

did the other 90 plants which were not responsive in the test system. The positive control (TPA) gave about 32% positive EA cell at concentration of 10 ng/ml.

Effect of plant extract concentration on EBV EA induction - The plant extracts that showed activity in the EA induction assay were further tested to a three-day treatment of Raji cells with various concentrations of the extract, ranging from 25 to 2,500 ng/ml (Fig. 1). In the presence of n-butyrate, all 8 plant extracts induced EA in a synergistic manner. It should be noted that *Euphorbia tirucali, E. splendes, Jatropha podagrica, Excoecaria agallocha* and *Codiaeum variegatum* showed their optimal activity at 1,000 ng/ml whereas

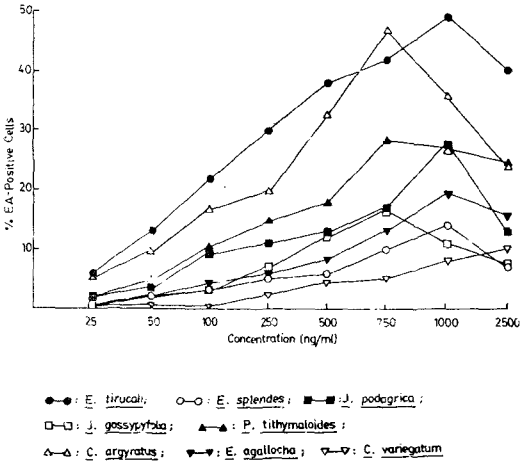

Fig. 1. Induction of EBV EA in Raji cells by plant extracts in the presence of 4 mM n-butyrate

Table 2. Activity of EA inducing principles in plant extracts

Plant Species	Relative Activity (RA)[a]
Euphoribia tirucali	94.2
E. splendes	28.3
Jatropha podagrica	52.8
J. gossypyfolia	30.1
Pedilanthus tithylamoides	54.7
Croton argyratus	88.6
Exocoecaria agallocha	35.8
Codiaeum variegatum	15
n-butyrate	5.6
TPA	100

TPA at a concentration of 10 ng/ml and n-butyrate at a concentration of 4 mM induces fluorescence in 32% and 1%, respectively of Raji cells.

The data are values relative to the TPA values.

$$RA = \frac{\%\ \text{EBV expression by compound}}{\%\ \text{EBV expression by TPA}} \times 100$$

All plants were assayed at their optimal concentration.

Jatropha gossypyfolia, Pedilanthus tithymaloides and Croton argyratus produced optimal inductionat 750 ng/ml with 17%, 30% and 17% of EA positive cells, respectively. The percentage of EA positive cells seemed to decrease with further increase in concentration, probably due to cytotoxic effects.

The activity of the extracts relative to TPA at 10 ng/ml is also shown in Table 2. It is observed that *Euphorbia tirucalli* shows a very high inducing activity closely followed by Croton argyratus, *Jatropha podagrica* and *Pedilanthus tithymaloides.*

Mouse Ear irritant activity - The plant extracts found to be positive for irritant activity are shown in Table 3. All the 8 plant extracts showed degree of irritation ranging from 1 to 3. The highest titre of 3 was obtained for *Croton argyratus.* None of the 90 plants which gave no response to EA induction had any irritant effect on mouse ear, except for *Annona murricata* (Annonaceae), whose irritant activity is similar to that of *Codiaeum, variegatum.*

Discussion

The inactive EBV genome in human B-lymphocytes can be activated to express virus-associated antigens by various chemical agents (Suguwara *et al.*, 1972; Luka *et al.*, 1979; Kawanishi and Ito, 1980). Tumour promoters, such as the phorbol esters, in the presence of low amount of sodium n-butyrate are capable of inducing the synthesis of the EA complex (Yamamoto *et al.*, 1981; Eliasson *et al.*, 1983). This has led to the development of a rapid and efficient assay to detect tumour promoter substances in nature (Ito *et al.*, 1981). By using this technique it was found that 8 species of 98 plants used in Malaysian traditional medicine tested positive for tumour promoting activity. All of the eight species belong to the family Euphorbiaceae. Moreover, all of these eight species caused skin irritation in the mouse assay.

The irritant effect of a substance on human skin has been taken as the first indication of the existence of tumour promoters in nature (Fujiki and Sugimura, 1987). However, it is difficult to differentiate between strong and weak tumour promoters by irritant test on mouse ear as compared to the activation of EBV-EA induction assay. Thus, two categories of assay systems have been proposed (Eliasson *et al.*, 1983). The first category consists of those test systems that equally induce both potent and weak promoters. These include irritant test on mouse ear (Hecker, 1971). The second category comprises in vitro tests that distinguish between strong and weak promoters. These include EA induction of Raji cells (Zeng *et al.*, 1983) and the adhesion of human promyelocytic leukemia cells, HL-60 (Hubermann and Callahan, 1979). Study on the effect of plant extracts on the induction of HL-60 cells adhesion is presently underway in our laboratory.

We found no evidence of EA activating prin-

Tabel 3. Effect of plant extracts on mouse ear after 24 hours incubation period

Plant extract	Number of mouse	Effect of plant extract and degree of irritation to mice ear							
		Neat (10 mg/ml)	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Euphorbia tirucali	a	3[a]	3	2	1	1	0	0	0
	b	3	2	2	1	1	0	0	0
	c	3	2	2	1	1	0	0	0
	d	3	2	1	1	0	0	0	0
E. splendes	a	1	0	0	0	0	0	0	0
	b	2	1	0	0	0	0	0	0
	c	2	1	0	0	0	0	0	0
	d	2	1	0	0	0	0	0	0
Jatropha podagrica	a	3	2	2	1	0	0	0	0
	b	2	1	1	0	0	0	0	0
	c	3	2	2	1	0	0	0	0
	d	2	2	1	0	0	0	0	0
J. gossypyfolia	a	3	2	1	0	0	0	0	0
	b	3	2	1	0	0	0	0	0
	c	2	1	1	0	0	0	0	0
	d	2	1	1	0	0	0	0	0
Pedilanthus tithylamoides	a	2	1	0	0	0	0	0	0
	b	2	2	1	0	0	0	0	0
	c	3	2	1	0	0	0	0	0
	d	3	2	1	0	0	0	0	0
Croton argyratus	a	3	3	2	2	1	0	0	0
	b	3	2	2	2	1	0	0	0
	c	3	2	2	1	0	0	0	0
	d	3	2	1	1	0	0	0	0
Exocoecaria agallocha	a	2	1	0	0	0	0	0	0
	b	2	1	0	0	0	0	0	0
	c	1	0	0	0	0	0	0	0
	d	2	1	0	0	0	0	0	0
Codiaecum variegatum	a	1	1	0	0	0	0	0	0
	b	1	0	0	0	0	0	0	0
	c	1	1	0	0	0	0	0	0
	d	1	1	0	0	0	0	0	0
TPA	a	4	4	4	4	3	3	3	3
	b	4	4	4	4	3	3	3	3
	c	4	4	4	3	3	3	3	3
	d	4	4	4	3	3	3	3	3

[a]Key: 0=no response; 1=25% irritant; 2=50% irritant; 3=75% irritant; 4=100% irritant.

ciple in the extrct of *Euphorbia hirta*, as reported for those from Hong Kong but the same plant species from Kenya (Africa) showed the presence of promoter activity (Ito *et al.*, 1983).

Similarly, the local *Codiaeum variegatum* was previously found to have no EA-activity (Yadav *et al.*, 1989), but in this study it was found to show a low level of EA induction. These observations indicate that similar plant species from different geographic localities may have qualitative and quantitative differences in their tumour promoting biological activity. It is interesting to note that *Codiaeum variegatum* used in the present study was collected from a plant grown in the vicinity of *Croton argyratus*, which shows a strong EA induction activity. It is possible that the EA activity may have been passively transferred via the soil. Indeed, Ito *et al.* (1983) noted that active diterpene esters were found in the soil underneath the plants which contain these substances and that these diterpenes could be passively passed to other plants in the surrounding area.

We noted that a high proportion of local medicinal plants of the family Euphorbiaceae possess the EBV-EA activating and irritant activities. The Euphorbiaceae which grow in warm and temperate climates have been used widely as folk remedies in East Africa (Kokwaro, 1976), Southern China (Ito, 1986) and Malaysia (Burkill, 1966). Epidemiological studies have shown increased prevalence of nasopharyngeal carcinoma and Burkitt's lymphoma (BL) in those areas where these plants are commonly used (Hirayama and Ito, 1981). It has been proposed that any inflammation in the mucosal area of the nasopharynx may lead to increased infiltration of the lymphatic cells (Moore *et al.*, 1974). The plant's tumour promoters when applied as medication may act synergistically with endogenously produced short-chain fatty acid, such as butyric acid, to activate the EBV genomes harboured in the lymphatic B cells. This process may initiate the viral replication cycle.

While traditional medication is used extensively by rural and urban Malaysians, often as an alternative to western medication, it is not clear whether the prevalence of some types of cancer is related to the use of such remedies. The present observations would suggest that it will be desirable to avoid the use of those plants which have been shown to contain tumour promoters.

Acknowledgements

The present study was supported by funds received from the University of Malaya's Research Grants Committee and from the Australian-Asean Biotechnology Research Grant Assistance Bureau. We thank Mrs. Irene Seow for her skilful technical assistance.

References

Burkill, I.H., *A Dictionary of the Economic Products of the Malay Peninsula.* Vols. 1 and 2. Ministry of Agriculture and Cooperatives, Kuala Lumpur, 1966.

Eliasson, E., Kallin, B., Pacerroyo, M., Klein, G., Fujiki, H. and Sugimura, T., Activation of the EBV-cycle and aggregation of human blood lymphocytes by the tumour promoters teleocidin, lyngbiatoxin A, aplysiatoxin and debromoaplysiatoxin. *International Journal of Cancer,* 31, 7-11 (1983).

Evans, F.J., *Naturally Occurring Phorbol Esters.* CRC Press Inc., Boca Raton, 1986.

Fujiki, H., Mori, M. Nakayasu, M., Terada, M, and Sugimura, T., A possible naturally occurring tumour promoter, teleocidin B from Streptomyces. *Biochemical and Biophysical Research Communications,* 9, 976-983 (1979).

Fujiki, H. and Sugimura T., New classes of tumour promoters: teleocidin, aplysiatoxin and palytoxin. *Advances in Cancer Research,* 49, 223-264 (1987).

Hecker, E., Isolation and characterization of the carcinogenic principles from croton oil. *Methods of Cancer Research,* 6, 439-484 (1971).

Hecker, E., Tumour promoters of the irritant diterpene ester type as risk factor of cancer in man. *Botanical Journal of Linnean Society,* 94, 197-219 (1987).

Hirayama, T. and Ito, Y., A new view of the eti-

ology of nasopharyngeal carcinoma. *Preventive Medicine*, 10, 614-622 (1981).

Hubermann, E. and Callahan, M.F., Induction of terminal differentiation in human promyelocytic leukemia cells by tumour promoting agents. *Proceedings of the National Academy of Sciences (USA)*, 76, 293-297 (1979).

Ito, Y., Yanase, S., Fujita, J., Hirayama, T., Takashima, M. and Imenaka, H., A short-term in vitro assay for promoter substances using human lympoblastoid cells latently infected with Ep stein-Barr virus. *Cancer Letters*, 13, 29-37 (1981).

Ito, Y., Yanase, S., Tokuda, H., Kishishita, M., Ohigashi, H., Hirota, M. and Koshimizu, K., Epstein-Barr virus activation by tung oil extracts of Aleurites frodii and its diterpene ester 12-0-hexadecanyol-13-acetate. *Cancer Letters*, 18, 87-95 (1983).

Ito, Y., Vegetable activators of the viral genome and the causaion of Burkitt's lymphoma and nasopharyngeal carcinoma, in M.A. Epstein and B.G. Achong (eds.). *The Epstein-Barr Virus: Recent Advances*, W. Heinemann Medical Books, London, 1986, pp. 208-236.

Kawanishi, M. and Ito, Y., Effect of short chain fatty acids on Epstein-Barr virus early and viral capsid antigen induction in P3HR1 cells. *Cancer Letters*, 11, 129-132 (1980).

Kokwaro, J.O., *Medicinal Plants of East Africa*. East Africa Literature Bureau, Nairobi, 1976, pp. 85-100.

Luka, J., Kallin, B. and Klein, G., Induction of Epstein-Barr virus cycle in latently infected cells by n-butyrate. *Virology*, 94, 228-231 (1979).

Moore, W.E.C. and Holdman. L.V., Fusabacterium, *in Bergey's Manual of Determinative Bacteriology*, Williams and Wilkins, *Baltimore*, 1974, pp. 404-408.

Norhanom, A.W., Yadav, M. and Singaram, S.P., A survey of antibody levels to Epstein-Barr virus in relation to early diagnosis of nasopharyngeal carcinoma in Malaysia. *Tropical Biomedicine*, 4, 25-31 (1987).

Perry, L.M. and Metzger, J.C., *Medicinal Plants of East and South East Asia. Attributed Proprties and Uses*. MIT Press, Cambridge, 1980, pp. 620.

Sugiwara, K., Mizuno, F. and Sato, T., Epstein-Barr virus associated antigens in nonporducing clones of human lymnphoblastoid cell lines. *Nature New Biology*, 239, 242-243 (1972).

Yadav, M., Ilham, M. and Norhanom, A.W., Epstein-Barr virus early antigen inductions in Raji cells by plants used in Malaysian traditional medicine. *Asean Journal of Clinical Sciences*, 9, 71-77(1989).

Yamamoto, H., Katsuki, T., Hinuma, Y., Hoshino, H., Miwa, M., Fujiki, H. and Sugimura, T., Induction of Epstein-Barr virus by a new tumour promoter, teleocidin, compared to induction by TPA. *International Journal of Cancer*, 28, 125-129 (1981).

Zeng, Y., Zhong, J.M., Mo, Y.K. and Miao, X.C., Epstein-Barr virus early antigen induction in Raji cells by Chinese medicinal herbs. *Intervirology*, 19, 201-204 (1983).

Zur Hausen, H., O'Neil, F.J. and Freese, U.K., Persisting oncogenic herpesvirus induced by the tumour promoter TPA. *Nature*, 272, 373-375(1978).

Zur Hausen, H., Bornkamm, G., Schmidt, R. and Hecker, E., Tumour initiators and promoters in the induction of Epstein-Barr virus. *Proceedings of the National Academy of Science (USA)*, 76, 782-785 (1979).

(Accepted 3 November 1995)