CHAPTER 2

LITERATURE REVIEW

2.1 MEDICINAL PLANTS

The use of medicinal plants as a source of relief from illness can be traced back over a millenium to written documents of the early civilizations in China, India and the Near East, but it is doubtless an art as old as mankind (Hamburger and Hostettmann, 1991). Even today, plant are the most exclusive source of drugs for the majority of the world's population. In industrialized countries, medicinal plants research has had its ups and downs during the last decades. But nonetheless, substances derived from higher plants constitute about 25% of prescribed medicines (Principe, 1989).

Medicinal plants are used basically in two different forms (Hamburger and Hostettmann, 1991) : (i) as complex mixtures containing a broad range of constituents (infusions, essential oils, tinctures, extracts); and (ii) as pure, chemically defined active principles. Pure compounds are generally employed when the active principles of a medicinal plants exhibit strong, specific activity and/or have a small therapeutic index, requiring accurate and reproducible dosage. On the other hand, the use of extracts/tinctures etc. is appropriate for plants exhibiting weaker and/or less specific pharmacological activities and if the active principles of a medicinal plant are yet unknown.

2.1.2 Anti-HIV Plants

The identification of the human immunodeficiency virus (HIV) as the causative agent of AIDS has stimulated the search for novel antiviral agents. Large screening programs for anti-HIV drugs are underway at the US National Institutes of Health and leading pharmaceutical companies. Two plants derived compounds have emerged from these screenings : castanospermine (**4**) and hypericine (**5**) (Figure 2.1). The tetrahydroxyindolizidine alkaloid, castanospermine (Hohenschutz, 1981) occures in the toxic chestnut-like seeds of the evergreen Australian tree *Castanospermum australe* A.Cunn et Fras. (Leguminosae). The compoud is a potent inhibitor of β-glucosidase, β-glucocerebrosinase and lysomal α- and β-glucosidases (Saul *et al.*, 1983). More recently, castanospermine was found to inhibit replication of HIV (Walker, 1987). This compound inhibits the enzyme α-glucosidase I and II which are of key importance in the production of glycoproteins contained within the envelope of the virus (Gruters, 1987). The anthraquinone derivative, hypericine, occurs in certain species of *Hypericum* (Guttiferae), such as *H. perforatum* L. Hypericin has anti-retroviral activity. It inhibits the propagation of Friend leukaemia virus both *in vitro* and *in vivo.* A single dose of hypericin can prolonge survival of FLV-infected mice (Meruelo, 1988). Hypericin is being further developed by the Weizman Institute in Israel (Palca, 1991).

2.1.3 Anti-Malarial Plants

Malaria is still the most important tropical disease and the number of clinical cases is estimated to about 200 million annually. Resistance of *Plasmodium* strains to currently used anti-malarial drugs is a serious treat. First screening programmes aimed at new anti-malarial drugs of plant origin were initiated after World War II. The most promising anti-malarial compound discovered so far is artemisinin (qinghaosu, **6**) (Figure 2.1), isolated in 1972 by Chinese scientists from the medicinal plant qinghao (*Artemisia annua* L., Asteraceae). Qinghao has been used for over 2 000 years in China as a febrifuge and in malaria therapy (Liu *et al.*, 1979). Artemisinin is a sesquiterpene lactone, with an endoperoxide group essential for its activity. This compound represents a completely new chemical class of anti-malarial compounds with a high level of blood schizontocidal activity against *Plasmodium* strains resistant to all known antimalarials. However, due to highly lipopholic nature of artemisinin, there are problems to its administration as a drug. A series of derivatives have been synthesized (Klayman, 1985). Among the most active of these compounds are artemether (**7**), arteether (**8**) and sodium artesunate (**9**) (Figure 2.1). Artemether has undergone clinical trials in China and Burma (Woerdenberg *et al.*, 1990). Preclinical studies on arteether are presently being performed. Sodium artesunate is water-soluble and has been clinically tested for the intravenous treatment of *P. falciparum* infections.

2.1.4 Anti-Spasmodic Plants

In the search for novel plant-derived cardiovascular drugs, two Indian research groups at Hoecsht India and at the Central Drug Research Institute in Lucknow independently discovered the blood pressure lowering and antis-spasmodic effect of *Coleus forskolii* Briq. (Lamiaceae). Both teams subsequently isolated the active principle foskolin (**10**) (Figure 2.1) (Tandon *et al.*, 1971). This compound is a potent stimulator of adenylate cyclase activity. Foskolin has, therefore, been extensively used to investigate the physiological consequences of increased intracellular cAMP. The toxicity of foskolin is low. Clinical studies have so far focused on cardiovascular and bronchospasmolytic effects and on tumour-induced human platelet aggregation and pulmonary tumour colonization in mice characterized foskolin as a potential antimetastatic agent (Agrawal and Parks, 1983).

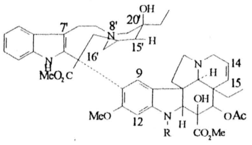
2.1.5 Anti-Asthmatic and Anti-Tussive Plants

The maidenhair tree (*Ginkgo biloba* L., Ginkgoaceae) is an old Chinese medicinal plants first mentioned in a medicinal book in 2 800 B.C. and is still part of modern Chinese medicine as an anti-asthmatic and anti-tussive drug. In Europe, first pharmaceutical specialities containing *Ginkgo* extracts were commercialized in the 1960s. Indications for these preparations are various disorders of peripheral blood circulation, and adjuvant treatment of the sequels of cerebral ischemia. The clinical efficacy of *Ginkgo* extracts was for a long time ascribed to the phenolic constituents (flavonol glycosides, biflavonoids). Only a few years ago was it discovered that the therapeutically-used extract GBE 761 specifically antagonizes platelet aggregation induced by the platelet activating factor (PAF) (Braquet *et al.*, 1985). Four compounds responsible for the inhibitory effect were subsequently isolated and identified as ginkgolides A (**11**), B (**12**), C (**13**) and M (**14**) (Figure 2.1) (Braquet, 1989). Besides a specific inhibition of PAF induced platelet aggregation, ginkgolides B antagonizes thrombus formation *in vivo* and also produces thrombolysis. Ginkgolides also exert a protective effect against bronchoconstruction induced in asthmatic patients. A synergic effect of ginkgolide B with immunosuppressant drugs has been observed in the supression of cell mediated graft rejection. In various models of ceberal ischaemia, ginkgolides reduced hypoxic damage as well as lesions (Braquet and Hosford, 1991).

2.1.6 Anti-Fertility Plants

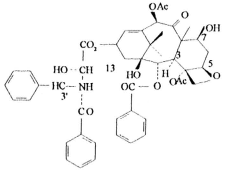
Birth control is a major issue in developing countries with rapid growing populations. Countries such as India and the People's Republic of China have important research programs devoted to fertility regulation with plant derived drugs (Pei-gen and Nai-gong, 1991). The dimeric sesquiterpene gossypol (**15**) (Figure 2.1) in the seeds of *Gossypium* species (Malvaceae) is certainly the most interesting compound with such an activity. The contraceptive effect of gossypol was discovered through the observation of subnormal fertility in rural communities in China where crude cottonseed oil was used for preparing food. Gossypol has been tested in China in more than 8 800 healthy men and the overall efficacy was 99.89%. Several undesirable side effects have rendered the use of gossypol as male contraceptive impracticable, in particular the irreversible caused by prolonged administration of the drug (Pei-gen and Nai-gong, 1991).

Figure 2.1 : Chemical structures of some active principles isolated from medicinal plants

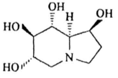


1 Me
2 CHO

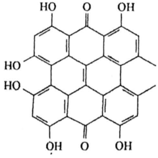
1 vinblastin
2 vincristine



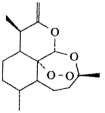
3 taxol



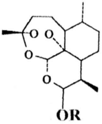
4 castanospermine



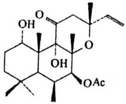
5 hypericine



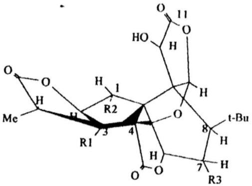
6 artemisinin



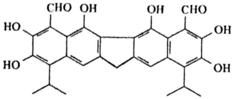
R	
Me	7 **artemether**
CH_2Me	8 **arteether**
$COCH_2CH_2CO_2Na$	9 **artesunate**



10 foskolin



R1	R2	R3	
OH	H	H	11 **ginkgolide A**
OH	OH	H	12 **ginkgolide B**
OH	OH	OH	13 **ginkgolide C**
H	OH	OH	14 **ginkgolide M**



15 gossypol

Table 2.1 : Some medicinal plants used as active principles in traditional medicine

Plant	Family	Compound	Activity	References
1. *Caranthus roseus* G.Don	Apocynaceae	Vinblastine Vincristine	Anti-tumour	Simpson and Ogorzaly, 1986
2. *Taxus brevifolia* Nutt.	Taxaceae	Taxol	Anti-tumour	Reuter, 1988
3. *Castanospermum australe* A. Cunn et Fras	Leguminosae	Castanospermine	Anti-HIV	Hohenschutz *et al.*, 1986 Saul *et al.*, 1983 Walker, 1987 Gruters, 1987
4. *Hypericum perforatum*L.	Guttiferae	Hypericine	Anti-retroviral	Murelo, 1988 Palca, 1991
5. *Artemisia annua* L.	Asteraceae	Artemisinin	Anti-malarial	Liu *et al.*, 1979
6. *Coleus forskolii* Briq	Lamiaceae	Foskolin	Anti-spasmodic	Tandon *et al.*, 1971 Agrawal and Parks, 1988
7. *Ginkgo biloba* L.	Ginkgoaceae	Ginkgolides A Ginkgolides B Ginkgolides C Ginkgolides M	Anti-asthmatic Anti-tussive	Braquet *et al.*, 1985 Braquet *et al.*, 1989 Braquet, 1991
8. *Gossypium sp*	Malvaceae	Gossypol	Anti-fertility	Pei-gen and Nai-gong, 1991

2.2 The Ebenaceae

The Ebenaceae are trees or shrubs, often with blackish bark, widespread in tropical and subtropical regions of both the Old and the New World, with only a few species extending into temperate climates (Cronquist, 1981). According to Cronquist (1981), the Ebenaceae are classified within the order of Ebenales. The Ebenales are woody plants, which comprises of five families and about 1 700 species. This includes the Sapotaceae with about 800 species, which make up nearly half of the order, and the Ebenaceae with about 450 species, making up another quarter. The Symplocaceae have about 300 species, the Styracaceae about 150 and Lissocarpaceae only two. One of the features of this order is the indefinite number of the floral parts especially the stamens which are often numerous arranged in several whorls (Keng, 1981).

The family Ebenaceae consists of five genera and 450 species. The great bulk of the family is belongs to the single genus *Diospyros* with about 400 species. The other genera are *Euclea*, *Rhaphidanthe*, *Royena* and *Tetraclis* (Cronquist, 1981). The family is the source of several economically important woods with notably ebony, the hard, very heavy black heartwood of *D. ebenum* Konig. and related species. The fruit of some species are edible (Cronquist, 1981). Thus, the family Ebenaceae are distinguished as follows : Trees, never vast, very rarely shrubs; bark usually black. Leaves alternate, entire, usually coriaceous. Flowers unisexual in axillary or extra-axillary cymes sometimes on the trunk. Calyx inferior, gamosepalous with 3 to 7 lobes accrescent. Corolla gamopetalous, white or yellow, small lobes 3 to 7. Stamens in 1 row as many as corolla-lobes or 2 or more times as many; filaments shorter than anthers, free or not; anthers linear; in female flowers; as staminodes or absent. Ovary superior (abortive or 0 in males); styles 2- to 8-cells as many or twice; ovules twice as many. Fruit a coriaceous or fleshy berry, often large. Calyx as base enlarged and often woody. Seeds oblong, albuminous.(Ridley, 1967).

2.3 The Genus *Diospyros* Linn.

Diospyros is a large genus of trees and shrubs of the family Ebenaceae. The genus consists of about 400 species which is nearly three quater of the family (Cronquist, 1981). They are found throughout the tropics and as well as in warm and temperate regions (Burkill, 1966). It grows well in areas with a monsoon climate from sea level to 800 m elevation (Verheij and Coronel, 1991). Thus, the genus *Diospyros* are described as follows: Trees, rarely shrubs. Leaves alternate. Calyx-lobes 4 to 5 (rarely 3), deep enlarged in fruit. Corolla tubular or campanulate, lobes 4 to 5, short. Stamens 4 to 64, usually 16, in males; staminodes 0 to 16 in females. Ovary 4- to 5-celled or imperfectly 8- to 20-celled; ovules 1, rarely 2 in each cell; styles and stigmas 1 to 4. Fruit globose, ellipsoid or conic-ovoid subtended by enlarged, sometimes woody calyx; flesh pulpy. Seed oblong, compressed, (Ridley, 1967).

Some species of *Diospyros* are fruit trees although most of them are ebony wood. The Malays call it *kayu arang* (charcoal wood) because of its colour. It has a more fancy name i.e *kayu sihangus* which refers to it as the wood that is endowed with burning. The ebony wood are used as a fancy wood. The following five species are sources of ebony in Malaysia and have been approximately one-eight of the total number of ebony yielding trees in the world. They are *D. buxifolia, D. scortechinii, D.graciliflora, D. clavigera* and *D. lucida* (Burkill, 1966).

The trees of some of *Diospyros* species possess poisonous properties which serve for narcotizing fish and lead to the use of such names as *tuba buah*, *mentuba* and *pokok ikan*.The poison is an irritant substances and that of some species will blister the skin (Burkill, 1966).

The Malays use ebony-wood medicinally. The strength of the wood to them suggests that it should give strength. They mixed a litle grated wood with coconut oil and rubbed it on a patient's abdoment (Ridley, 1967). The powdered wood is used for pains in the stomach, employing it both internally and externally. Another application of the magic of its strength is by placing of a little of the tree in the hole in the ground into which the centre of the post of the house is driven.

Certain tannin-colloids were found in the fruits. However, these colloids disappear at ripenness due to its combination with a mucillaginous substance which is plentiful produced in ripening. The tannin-colloids are metabolized as the fruit ripens in many species whose fruits are not edible. Fruits of others become very good to eat. It is also the tannin compound which makes the immature fruits are useful as a dye. The dye is best on silk, but is also extensively used to toughen fishing lines and nets, particularly in Indo-China and Thailand. A colloidal extracts of the fruits of some species is used as a paint on the bottom of boats and for water proofing paper umbrellas and fans (Burkill, 1966).

Malay names but no economic information have been recorded for the following species: *D .bilocularis*,Oliv., *nyatoh hitam* (black Palaquium), *kayu balum ijuk* (*balun hijau*); *D. cymosa* Ridl., *merangat; D. pubicarpa* Ridl., *hidung kelawar* (bat's nose; along with two allies); *D. subrhomboidea* King and Gamble, *hidung kelawar*; *D. wrayi* King and Gamble, *bulu-bulu* (in common with some other similarly hairy plants), *hidung kelawar*; *D. caliginosa* Ridl., *chakum, lampong* (both in common with *D. truncifolia*). *Bui* or *buhi* is a name used also for a *Diopyros* (Burkill, 1966).

2.3.1 *Diospyros graciliflora* Hiern.

Diospyros *graciliflora* Hiern. is a tree of moderate size. Its distribution was from Kedah to Malacca and was repoted to be in Java and Borneo (Burkill, 1966). It is distinguished as follows : Small tree 20 to 50 feet tall. Leaves thick membranous, narrow-elliptic caudate acuminate, base narrowed; nerves 5 pairs usually prominent and wide, inarching beneath; 2.5 to 4.5 in. long, 0.75 to 1.35 in. wide; petioles 0.1 to 0.2 in. long. Male flowers 0.5 in. long, few in sessile cymes puberulous. Calyx of 4 large ovate round lobes. Corolla narrow tubular, pale yellow, one-third longer; tubes with 4 lines of pubescence; lobes 4, deep long blunt. Stamen 8. Female like males and sometimes mixed with them. Fruit ellipsoid, 1 in. long, 0.75 in. wide, glabrous (Ridley, 1967).

According to Ridley (1967), this plant is called *kayu arang* or *kayu sihangus* which refers to the charcoal-like colours of the wood. The black wood was used for rulers and walking-sticks. In Pahang, it is recognized under the name of *ganding hutan* and was used as a protective medicine after child birth (Burkill, 1966). According to Pant and

Chaturvedi (1989), the leaves of this plant has been extinsively used in Indian medicine as an anti-hypertensive agent.

2.3.2 *Diospyros discolor*, Willd.

Diospyros discolor Willd. is one of the species from the genus of *Diospyros* which is widely distributed in primary and secondary forests at low and moderate altitudes (Burkill, 1966). It is indigenous to Philippines and has been introduced into other tropical countries (Verheij and Coronel, 1991).Its vernacular names is mabolo or velvet apple (English). In France, it is called Pommier velours whereas in Indonesia it called *buah mentega* or *bisbul* or *mabolo* (Burkill, 1966). In Philippines however, it is called *mabolo* or *kamong* which means hairy, referring to the hairy-fruit (Verheij and Coronel, 1991). The tree was taken to Calcutta in 1811 and to London 1822. It reached Java under the name *mabolo,* just as it reached Calcutta, but is not known when. Apparently, it reached Malaya independently, for the name *mabolo* was not kept, instead, it was called *buah mentega.* The tree was introduced into the Botanic Gardens, Singapore, in 1881 via Kew (Burkill, 1966).

The tree is commonly planted on road sides as they provide excellent shades. In the Philippines Islands, the timber is cut and marketed. The best combes in the Philippines markets are made from it (Burkill, 1966). According to Siddiqui *et al.*, (1988), the leaves of this plant are reputed as therapeutic agents in the treatment of swellings, leprosy and eye and skin disease. The leaves also possesses cardiotonic and anti-bacterial properties.

There are several races of fruits which are large and edible. The fruits in some are purplish red and in other copper-coloured. It is a purplish red race with sweet fruits which has been brought to Malaya. Other races have less sweet fruits. A seedles race has been detected (Burkill, 1966) but the smell of this fruit is a little mousy.

2.3.3 *Diospyros lanceifolia* Roxburgh.

Diospyros lanceifolia Roxburgh. Is a small tree or a large shrub found in Thailand, Sumatera and the Malay Peninsula (Ridley, 1967). It is commonly found in woods and rocky places by the sea. Thus it is distinguished as follows : A small tree or large shrub. Leaves coriaceous shining, lanceolate or oblong sub-acute or blunt, base round or narrow, glabrous; nerves 5 to 9 pairs, quite obscure; 1.25 to 4.5 in. long, 0.5 to 1.5 in. wide; petioles 0.2 to 0.4 in. long. Male flower sessile in small fascicles. Calyx 0.15 in. long, lobes broad, triangular, tomentose all over. Corolla narrow tubular, silky tomentose, yellow, 0.4 in. long; lobes oblong. Stamen 12 to 16 pairs. Females 1 to 2 together, sub-sessile. Calyx larger than in male. Fruit globose depressed, about 0.5 in. long, nearly glabrous; calyx broad, woody, flat, cup-shaped, 0.5 in. wide, angled, hardly lobed (Ridley, 1967).

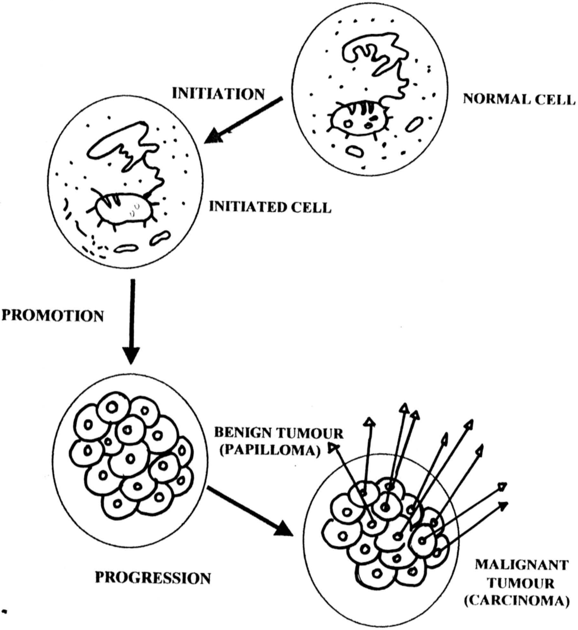
This plant is also called *kayu arang* (charcoal wood), *pokok melukut* (rice sifting plant), *pokok ikan mati* (tree of the dead fish), *nipis kulit*, *lenggadi*, sengkawas and *koguel*. In Sumatera, it is called *tuba pais* (Ridley, 1967). The fruit of *D. lanceifolia* is used as fish poison (Perry, 1985). The leaves of this plant possesses antibiotic as well as sedative properties (Dan and Dan, 1984). According to Ridley (1902), the timber of this plant is similar to that of *D. clavigera* but is rather darker.

2.4 Anti-Tumour Promoter Activity

It is now believed that almost all forms of carcinogenesis involved a multi-stage process and that each stage can be influenced by a variety of exogenous and endogenous factors. According to Berenbelum (1975), the experimental induction of malignant tumours in mouse skin can be divided into three stages called initiation, promotion and malignant progression (Figure 2.2). Recently, an additional stage called conversion has been defined.

Initiation is achieved by "subthreshold" treatment of skin with a carcinogenic agent of chemical, viral or physical nature. This treatment is thought to result in the formation of a limited number of "latent" tumour cells in the epidermis (Berenbelum, 1975). Promotion is brought about by repeated applications of certain agents such as the phorbol esters from croton oil, which in contrast to initiators do not exhibit transforming or mutagenic potential. Conversion can be operationally defined as a process which makes epidermis sensetive to promotion. In mouse skin, the initiation-conversion-promotion approach mainly leads to the development of benign tumours which are of monoclonal origin. Only after prolonged treatment do some carcinomas appear. The rate of progression from benign to the malignant state can be increased by additional applications of initiating carcinogens following the period of promotion. This indicates that progression may be due to an additional genetoxic effect (Berenbelum, 1975).

Figure 2.2 : Three-Stage Carcinogenesis Model (Berenbelum, 1975)



Recently, the potential anti-tumour promoter activity of several compounds have been investigated through several laboratory experiments. Saito *et al.* (1986), investigated the inhibitory effect of three anti-tumour promoters, namely butylated hydroxyanisole (BHA), butylated hydroxytoulene (BHT) and retinoic acid (RA) on 3-methylcholanthrene (3-MC)-induced transformation enhanced by TPA in BALB 3T3 cells. They found that BHA inhibited TPA-enhanced transformation in a dose-dependent manner but BHT did not. They also found that, among the three anti-tumour promoters tested, RA was the most effective inhibitor.

The effects of some compounds, which have been reported to inhibit tumour promoters *in vivo* (Ito *et al.*, 1981), on the induction of EA of EBV by TPA in Raji cells were examined by Saito *et al.* (1986). The inhibitors of the cascade process involving arachidonic acid, indomethacin, nordihydroguaiaretic acid, phenidone and *p*-bromophenacyl bromide which were effectively inhibited EBV EA induction by TPA. Two flavonoids, morin and kaempferol also inhibited EA induction. They also found that among antioxidants tested, calmodulin antagonist, N-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide and esculetin showed inhibitory effect of EA induction.

Nishino *et al.* (1986), has proved that glycyrrhetinic acid (GA) supressed tumour promoter effects. Since then, they have investigated some other oleane-type triterpenes which were chemically derived from oleanolic acid and hederagenin, *in vivo* and *in vitro*, against the action of tumour promoter, TPA. By *in vivo* experiment monitoring with TPA-induced stimulation of ^{32}Pi incorporation into phospolipids and *in vitro* test on skin tumour formation in mice initiated with 7,12-dimethylbenz[*a*]anthracene and promoted with TPA, 18β-olean-12-ene-3β, 28-diol (=erythrodiol), 18β-olean-12-ene-3β, 23,28-triol, 18α-olean-12-ene-3β, 28-diol and 18α-olean-12-ene-3β, 23,28-triol showed remarkable supressive effects. Both 18α-olean-12-ene-3β, 28-diol and 18α-olean-12-ene-3β, 23,18-triol were 100 times more effective than GA both *in vivo* and *in vitro*.

2.4.1 Anti-tumour Promoter Activity in Herbage Vegetables and Spice Tree Plants

The inhibition of EBV activation induced by HPA of 121 species (133 test-parts) of edible plants have been tested by Koshimizu *et al.*, (1988). The methanol extracts of 14 species of these plants strongly inhibited the activation. Among the 47 species (48 test-parts) of herbage vegetables including some species of spice tree plants, seven species were found to strongly inhibited the activation. They were *Colocasia esculenta* (taro-stem), *Lactuca sativa* L. var. *crispa* (curled lettuce), *Brasicca campestris* (field mustard-budding flower), *B. oleracea* L. var. *botrytis* (cauliflower), *Zanthoxylum piperitum* (Japanese pepper-flower), *Petroselinum sativum* (parsley) and *Zingerber officinale* (ginger-leaf sheath). A green perilla (*Perilla frustescens* Britt. var. *acuta*) and Japanese parsley (*Oenanthe javanica*) were found moderately active whereas a chinese mustard (*Brasicca chinensis*), dittany of crete (*Origanum vulgare*), chiboul (*Allium fistulosum* var. *caespitosum*), bracken (*Pteridium aquilinum*) and stone parsley (*Cryptotaenia japonica*) were found weakly active.

2.4.2 Anti-Tumour Promoter Activity of Medicinal Plants

Today, plants are the most exclusive source of drugs for the majority of world's population. Over 35 000 plant species have been screened by the National Cancer Institute (NCI) of the United States for their anti-tumour activity from 1957 to 1981 (Suffness and Douros, 1982) and is currently in the process of acquiring some 20 000 tropical species from Latin America, Africa and Southeast Asia (Cassady, 1990).

Plants contain several natural products from the class of alkaloids, terpenoids and phenolic substances including flavonoids and coumarins. Classes of anti-tumour promoter compounds are described below.

(i) Terpenoids

Acylphloroglucinol monoterpene and Acylphloroglucinolsesquiterpene

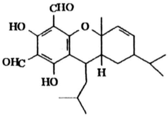
Twelve euglobal compounds having novel acylphloroglucinol monoterpene structures (or -sesquiterpene) structures were isolated from leaves and flower buds of *Eucalyptus globulus* Labill. (Takasaki *et al.*, 1990). These compounds which showed anti-inflammatory activity in a screening test using chick embryo and having strong inhibition of exuberant granulation, were tested for their inhibitory activities using a short-term *in vitro* assay of EBV EA activation in Raji cells induced by TPA. Among these compounds, euglobal-III (23) showed strong inhibitory activity, and euglobal-Ib (18) and -IIa (20) exhibited highly significant activities at 1 000 mol TPA ratios, respectively. Euglobal-Ic (19) was also noted for its activity at 1 000 mol/TPA and 100 mol/TPA ratios. Euglobal-Ia_1 (16) and -Ia_2 (17) had considerable activity at 100 mol ratio per TPA. Euglobal-V

(26) was not effective at low concentration (under 100 mol/TPA ratio), while euglobal-IIb (21), -IVa (24), -IVb (25) and euglobals having isovaleryl group on the aromatic ring, e.g. euglobal-IIc (22) and -VII (27) showed weak activity. Structures of all compounds isolated were shown in Figure 2.3.

Further investigation of other euglobals led to the isolation of three new compounds, euglobal-G1 (28), -G3 (29) and -G4 (30) (Figure 2.4) from the leaves of *E. grandis* W.Hill. These three compounds exhibited strong inhibitory activity on the EBV activation with 100% inhibition at 1x10 mol ratio compound/TPA (Takasaki *et al.*, 1990).

Kokumai *et al.* (1991), had done a continuing chemical and biological study from the juvenile leaves of *E. tereticornis* Sm. A new euglobal namely euglobal T1 (31) (Figure 2.5) have been isolated. However, this compound showed weak inhibitory activity on EBV EA activation in Raji cells.

Figure 2.3 : Chemical structure of euglobals isolated from the leaves and flower buds of *E. globulus*



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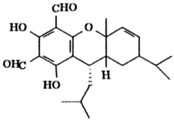

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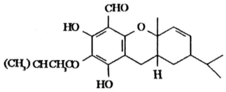

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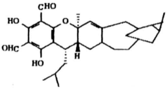

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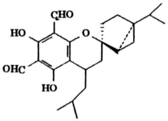

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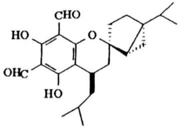

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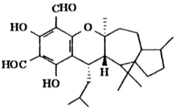

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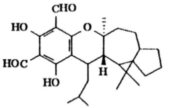

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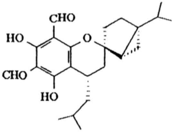

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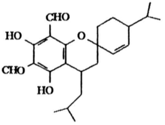

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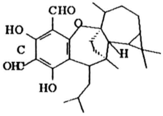

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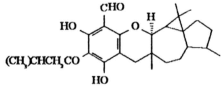
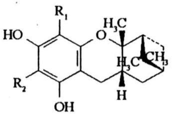


27

16 Euglobal-Ia	**22 Euglobal-IIc**
17 Euglobal-Ia$_2$	**23 Euglobal-III**
18 Euglobal-Ib	**24 Euglobal-IVa**
19 Euglobal-Ic	**25 Euglobal-IVb**
20 Euglobal-IIa	**26 Euglobal-V**
21 Euglobal-IIb	**27 Euglobal-VII**

Figure 2.4 : Chemical structures of the euglobals isolated from the leaves of *E. grandis*



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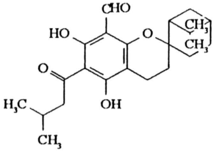

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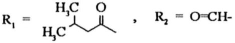

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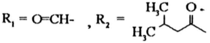
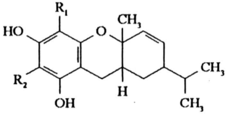
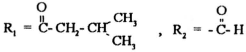


28 Euglobal - G1
29 Euglobal - G3
30 Euglobal - G4

Figure 2.5 : Chemical structures of euglobal T1 isolated from the juvenile leaves of *E. tereticornis*





31 Euglobal - T1

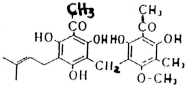
Phloroglucinol and phloroglucinol derivatives

Arisawa *et al.* (1991), have been studying constituents of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae) and have reported the isolation, identification and structure elucidation of several new phloroglucinol derivatives with their anti-tumour and anti-herpetic activities. They found that the methanol (MeOH) and chloroform ($CHCl_3$) extracts of pericarp of *M .japonicus* proved to have potent anti-tumour promoter activity in the screening test *in vitro*, i.e., both extracts showed 100% inhibitory effects on TPA-enhanced 3H-choline incorporation into phospholipids of C3H 10T1/2 cells *in vitro* at 50 μg/ml.

Seven isolated phloroglucinol derivatives from the $CHCl_3$ extract of *M. japonicus*, mallotojaponin (**32**), mallotolerin (**33**), mallotochromene (**34**), mallotophenone (**35**), mallotochromanol (**36**), isomallotochromanol (**37**) and 2,6-dihyroxy-3-methyl-4-methoxyacetophenone (**38**) and two phloroglucinol derives from mallotojaponin (**32**), namely mallotochroman (**39**) and isomallotochromene (**40**) were also tested for potential anti-tumour promoter activity. All of them markely inhibited tumour promoter stimulated 3H-choline incorporation into phospholipids of C3H 10T1/2 cells. (Structures of all compounds were shown in Figure 2.6).

Anti-cancer effect of compound **32** on mouse L 5178Y Leukemia *in vivo* and combined treatment of compound **32** and OK-432 on mouse L 5178Y Leukemia and Ehrlich ascites carcinoma were also done. Compound **32** showed significant cytotoxicity with all the tumour cell lines *in vitro*, and potent antileukemic activity *in vitro* was observed.

Figure 2.6 : Chemical structures of phloroglucinol derivatives from chloroform extract of *M. japonicus*



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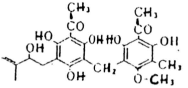

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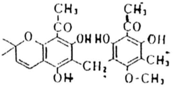

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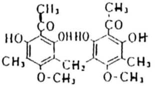

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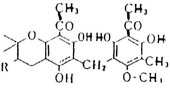
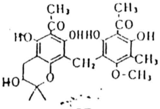


36 R=OH

39 R=H



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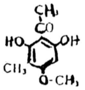

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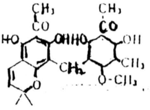


40

32 **mallotojaponin**
33 **mallotolerin**
34 **mallotochromene**
35 **mallotophenone**
36 **mallotochromanol**
37 **isomallotochromanol**
38 **2, 6-dihydroxy-3-methyl-methoxyacetopheneone**
39 **mallotochroman**
40 **isomallotochromene**

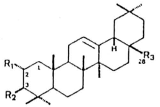
Triterpenoids

Konoshima *et al.* (1987), have isolated and identified eight triterpenes from the bark of *Euptelea polyandra* Sieb. Zucc (Eupteliaceae) with anti inflammatory action and carried out a primary screening of the triterpene and their derivatives using their posssible inhibitory effects on EBV EA activation in Raji cells. Twelve oleane-type triterpene (40-52) and five lupane-type triterpenes (53-57) (Figure 2.7) were tested using a short-term *in vitro* assay on EBV EA activations in Raji cells induced by TPA.

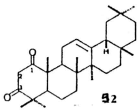
In the oleane-type triterpenes, 3-O-acetyloleanolic acid (42) and 3-O-acetylerythradiol (47) showed remarkable inhibitory effects than oleanolic acid (41) and erythrodiol (46), whereas maslinic acid (49) having a 2α-OH group, 2,3-di-0-acetylmaslinic (50) and 1,3-dioxo-olean-12-ene (52) exhibited higher inhibitory effects than triterpene having a 3β-OH group. On the other hand, these remarkable inhibitory effects were not found with oleanolic aldehyde (44) and its acetate (45).

In the series of lupane-type triterpenes, only 3-O-acetyl betulinic acid (54) showed significant inhibitory activity (complete inhibition of activation even at $1x10^3$ mol ratio), whereas the other lupane-type triterpenes (53, 55, 56 and 57) showed no activity at all.

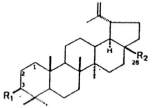
Figure 2.7 : Chemical structures of oleane- and lupane-type triterpenes isolated from the bark of *E. polyandra*



	R_1	R_2	R_3
41	-H	-OH	-COOH
42	-H	-OAc	-COOH
43	-H	-OH	-COOMe
44	-H	-OH	-CHO
45	-H	-OAc	-CHO
46	-H	-OH	$-CH_2OH$
47	-H	-OAc	$-CH_2OH$
48	-H	-OAc	$-CH_2OAc$
49	-OH	-OH	-COOH
50	-OAc	-OAc	-COOH
51	-OH	-OH	-COOMe



52



	R_1	R_2
53	-OH	-COOH
54	-OAc	-COOH
55	-OH	-COOMe
56	-OH	$-CH_2OH$
57	-OAc	-Me

41 **oleanolic acid**

42 **3-O-acetyloleanolic acid**

43 **metil oleanolic acid**

44 **oleanolic aldehyde**

45 **oleanolic acetate**

46 **erythrodiol**

47 **3-O-acetylerythrodiol**

48 **3-O-acetylerythroetil**

49 **maslinic acid**

50 **2, 3-di-O-acetylmaslinic acid**

51 **metil maslinic acid**

52 **1, 3-dioxo-olean-12-ene**

53 **betulinic acid**

54 **3-O-acetyl-betulinic acid**

55 **metil betulinic acid**

56 **betulin**

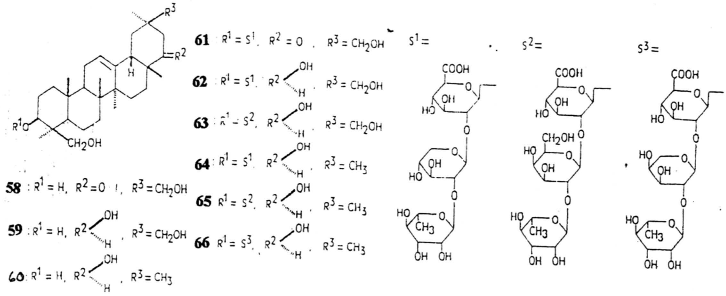
57 **3-O-acetyl-metil betulinic acid**

Triterpenoid Saponins

Konoshima *et al.* (1989), has described the structure elucidations and the inhibitory effects on EBV activation by the tumour promoter, TPA, of six triterpenoid saponins and three saponins isolated from the knots of *Wistaria brachybotrys* Sieb. et Zucc. (Leguminosae). The triterpenoid saponins were identified as wistaria sapogenol A (**58**), wistariasapogenol B (**59**), wistariasaponin A (**60**), wistariasaponin B_1 (**61**), wistariasaponin B_2 (**62**) and wistariasaponin C (**63**), whereas the saponins isolated were soyasapogenol B (**64**), soyasaponin I (**65**) and soyasaponin II (**66**). The structure of all compounds isolated were shown in Figure 2.8.

Wistariasapogenol A (**58**), soyasapogenol B (**64**), wistariasaponin A (**60**), wistariasaponin C (**63**), soyasaponin I (**65**) and soyasaponin II (**66**) exhibited notable inhibitory effects at 1×10^3 mol ratio. The degrees of inhibitory activity of **64**, **60**, **65** and **66** were compared to that was found with oleanolic acid, a known inhibitor of EBV activation (Konoshima *et al*, 1987). Compound **60** and **66** showed significant inhibitory effect (20-30%) even at low dose (1×10^2 mol ratio). On the other hand, such effect were not found with 59, 61 and **62**.

Figure 2.8 : Chemical structures of saponin isolated from the knots of *W. brachybotrys*

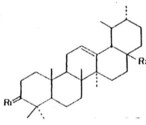


58	**wistariasapogenol A**	63	**wistariasaponin C**
59	**wistariasapogenol B**	64	**soyasapogenol B**
60	**wistariasapogenol A_1**	65	**soyasaponin 1**
61	**wistariasaponin B_1**	66	**soyasaponin II**
62	**wistariasaponin B_2**		

Triterpene Carboxylic Acid

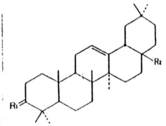
Two triterpene carboxylic acids, ursolic acid (67) and oleanic acid (68) (Figure 2.9) have been isolated from an anti-inflammatory medicinal plant, *Glechoma hederaceae* L., as inhibitors of TPA induced EBV activation in Raji cells (Ohigashi *et al.*, 1986). Both compounds were tested against inhibitory effect on tumour promotion by TPA *in vivo.* They found that ursolic acid and oleanic acid inhibited effectively the tumour promotion in mouse skin and the activities were comparable to that of a known inhibitor of tumour promotion, retinoic acid (Saito *et al.*, 1986). They also suggested that the role of oleanic acid for inhibitory action on tumour promotion differs slightly from those retinoic acid and oleanic acid as ursolic acid was more effective on a single application before initial TPA-treatment than on a continuous application before each TPA-treatment, while oleanic acid and retinoic acid were ineffective in the same treatment.

Figure 2.9 : Chemical structures of ursolic and oleanic acid from *G. hederaceae*



$R_1 = \beta$-OH, H $R_2 = COOH$

67 ursolic acid



$R_1 = \beta$-OH $R_2 = COOH$

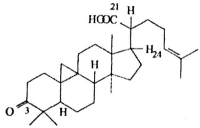
68 oleanic acid

Cycloartenoid Triterpene

A new cycloartenoid triterpene, 3-oxo-24-cycloarten-21-oic acid (1) have been isolated by Nishizawa *et al.*(1989), from the leaves of *Lansium domesticum* (Meliaceae) (Figure 2.10). Seventeen chemical derivatives (**70-86**)(Figure 2.11) of this compound were also prepared by simple chemical transformation of compound **69**. All of these compounds were tested for their anti-tumour promoter activity using a short term *in vitro* assay of EBV EA activation in Raji cells induced by TPA.

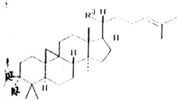
It was found that **69**, **72**, **73**, **74**, **75**, **76**, **77**, **78**, **80**, **81**, **83**, **84** and **86** showed significant activities with complete inhibition at sample concentration of 0.32 nM. At the same concentration, compounds **70**, **71**, **79** and **85** gave more than 90% inhibition.

Figure 2.10 : Chemical structure of a cycloartenoid triterpene isolated from the leaves of *L. domesticum*

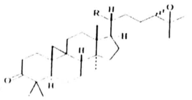


69

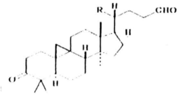
Figure 2.11 : Chemical structures of derivatives of 3-oxo-24-cycloarten-21-oic acid



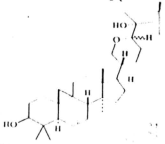
70 $R^1, R^2 = O$		$R^3 = COOCH_3$
71 $R^1 = OH$	$R^2 = H$	$R^3 = COOCH_3$
72 $R^1 = H$	$R^2 = H$	$R^3 = COOCH3$
73 $R^1 = OH$	$R^2 = H$	$R^3 = COOH$
74 $R^1 = OH$	$R^2 = H$	$R^3 = CH_2OH$
75 $R^1 = H$	$R^2 = OH$	$R^3 = CH_2OH$
76 $R^1 = OAc$	$R^2 = H$	$R^3 = CH_2Oac$
77 $R^1 = OAc$	$R^2 = H$	$R^3 = COOH$
84 R^1, R2 = O		$R^3 = CHO$
85 $R^1 = OH$	$R^2 = H$	$R^3 = CHO$
86 $R^1, R^2 = O$		$R^3 = CH_2OH$



78 R = COOH (1:1 mixture)
79 R = $COOCH_3$ (1:1 mixture)



82 R = $COOCH_3$
83 R = CHO

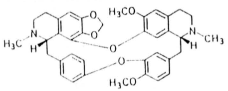


80 less polar isomer
81 polar isomer

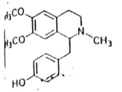
(ii) Alkaloids

Fujitani *et al.* (1990), have tested thirty bisbenzylisoquinoline alkaloids and fifty benzylisoquinolines including related non-natural synthetic compounds for their inhibitory acvtivities on TPA-induced EBV activation in Raji cells. Of the bisbenzylisoquinoline alkaloids, cepharanthine (87) and secocepharanthine (88) showed inhibitory activities. Among the benzylisoquinolines, armepavine (89) and laudanosine (90) exhibited highly significant activities. (Structures of all compounds were shown in Figure 2.12).

Figure 2.12 : Chemical structures of bisbenzylisoquinoline alkaloids



87



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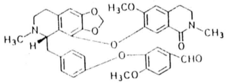

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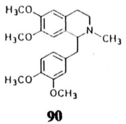


90

87	**cepharanthine**	**89**	**armepavine**
88	**secocepharanthine**	**90**	**laudanosine**

(iii) Phenolic Substances

The inhibitory effects of (-)epigallocatechin gallate (EGCG), the main constituent of Japanese green tea on tumour promotion with two tumour promoters, teleocidin and okadaic acid (a non-TPA-type tumour promoter), have been studied by Fujiki *et al.* (1992).

Initiation was carried out by a single application of 50 μg DMBA and tumour promotion was achieved by application of 2.5 μg teleocidin twice a week. Five milligrams EGCG, applied topically before each treatment with telocidin, reduced the percentage of tumour-bearing mice from 53 to 13% in week 25 and the average number of tumours per mouse from 2.1 to 0.1. ECGC treatment using okadaic acid, completly inhibited tumour promotion on mouse skin. They also suggested on drinking tea in large amounts for cancer prevention in the general population. For the high risk group of cancer of the liver and gastrointestinal track, the use of EGCG should be further investigated.

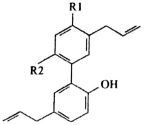
__Neolignans__

The bark of *Magnolia officinalis* Rehd. et Wils. (Magnoliaceae) has been used in Chinese and Japanese folk medicine for the treatment of bronchitis and emphysema (Tokuda *et al.*, 1991). This crude drug is one of the important components of the Kampo prescription. The methanol (MeOH) extract of the bark of *M. officinalis* was found to show significant inhibitory effect on EBV activation on Raji cells at low dose i.e. 100% inhibition of activation at 100 μg/ml and 39.3% inhibition of activation even at 1 μg/ml (Konoshima *et al.*, 1991).

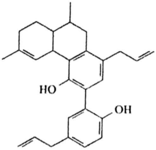
Bioassay-directed fractionation of the active extract led to the isolation and characterization of three neolignans : magnolol (91), honokiol (92) and monoterpenylmagnolol (93) (Figure 2.13) as inhibitory principles of EBV EA activation. Among these neolignans, magnolol exhibited a most significant inhibitory activity i.e. 70% inhibition at $1x10^2$ mol ratio of inhibitor/TPA and 40% inhibition even at 1x10 mol ratio. Honokiol and monoterpenylmagnolol exhibited strong inhibitory activities only at high doses ($1x10^3$ and $5x10^2$ mol ratios). The inhibitory activities of magnolol and honokiol were more than 10 times higher than those of retinoic and glycyrretinic acids which are known as strong anti-tumour promoters (Tokuda *et al.*, 1986).

On the basis of the results of the *in vitro* assay, the effect of the extract of *M. officinalis* and magnolol on two stage carcinogenesis *in vivo* were investigated. Both the MeOH extract (50 μg) and magnolol (85 nmol) when applied continuously before each TPA treatment, delayed the formation of papillomas in mouse skin as compared with the control experiment only with TPA.

Figure 2.13 : Chemical structures of the neolignans isolated from the bark of *M. officinalis*



91 R1= H, R2= OH
92 R1= OH, R2= H



93

91-magnol
92honokiol
93 monoterpenylmagnolol

Quinones

Anthraquinones and naphthoquinones occurs widely in the plant kingdom and in crude drugs. These quinones may have an important role in the biological activities of many plants and crude drugs. Koyama *et al.* (1989), have isolated anthraquinones, naphtoquinones, azaanthraquinones, azafluorenones and their related compounds in the family Annonaceae and carried out an evaluation of the inhibitory activities of these fifty one (51) quinones on EBV EA activation in Raji cells. They have found that three out of eighteen anthraquinones tested (labelled as 102, 103 and 109 (Figure 2.14) exhibited very strong inhibitory activities at low doses ($1x10^2$ mol ratio/TPA). Emodin (101), damnacanthol (106), 1,3-dihydroxy-2-methoxymethyl-anthraquinone (107) and damnacanthal (110) (Figure 2.14) showed the inhibitory effect only at high doses ($1x10^3$ mol ratio/TPA). Quinizarin (94), alizarin (95) and sennoside B (111) showed no activity at all. Anthraquinones in which hydroxyl groups were evenly distributed in phenyl rings, 1,8-dihydroxy anthraquinone (96), anthrarufin (97), anthraflavic acid (98) and quinalizarin (100) exhibited greater inhibitory effect than 94, 95 and 99 in which hydroxyl groups were unevenly distributed on phenyl rings.

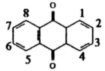
Sixteen compounds from the naphtoquinones series were also tested for their anti-tumour promoter activities. Naphtazalin (114), shikonin (122) and the furanoquinones (124-127) (Figure 2.15) exhibited very strong inhibitory activities even at low doses (1x10 mol ratio/TPA) and these activities were more than ten times higher than those of the active anthraquinones and azaanthraquinones. Also these inhibitory activities are more than 100 times higher than those of glycyrrhetinic and retinoic acid, which are known as inhibitors of

EBV EA activation and tumour promotion (Ohigashi *et al.*, 1986). Thiophenonaphtoquinone (127) exhibited lower inhibitory effect than furanonaphtoquinones (124-126), and vitamin K_1 (123) showed no activity at all even at high dose ($1x10^3$). From the comparisons of the inhibitory effects of 114, 122 and 120 with those of 112, 113, 115, 116 and 117, it was also deduced that the hydroxyl groups at C-5 and C-8 on the naphtoquinone skeleton enhanced these inhibitory effects.

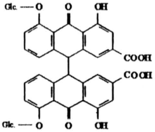
In the azaanthraquinones series, compounds (129), (130), (134), (136), (137) and (138) (Figure 2.16) exhibited significant inhibitory activities at $5x10^2$ mol ratio, whereas these significant inhibitory effects were not found with cleistopholine (128), (131), (132), (133) and (135). For the three azafluorenones (142-144) (Figure2.16), significant inhibitory effects on EBV EA activation were not shown even at $5x10^2$ mol ratio.

From the result of a binding assay, it was deduced that the active quinones 102, 103, 122, 125, 130 and 138 showed no effect on [3H]-TPA binding to the TPA receptor, while TPA significantly inhibited it. These results indicated that quinones may act at some point after the binding of the tumour promoters to the receptors.

Figure 2.14 : Chemical structures of some anthraquinones isolated from the plants of family Anonaceae

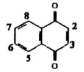


Compound	Substituents						
	C-1	C-2	C-3	C-4	C-5	C-6	C-8
94 (quinizarin)	OH	-	-	OH	-	-	-
95 (alizarin)	OH	OH	-	-	-	-	-
96	OH	-	-	-	-	-	OH
97 (anthrarufin)	OH	-	-	-	OH	-	-
98 (anthraflavic acid)	-	OH	-	-	-	OH	-
99 (purpurin)		OH	-	OH	-	-	-
100 (quinalizarin)	OH	OH	-	-	OH	-	OH
101 (emodin)	OH	-	OH	-	-	Me	OH
102	O-CH_2-O	-	-	-	OH	-	-
103	O-CH_2-O	-	-	-	OMe-	-	-
104	O-CH_2-O	-	-	-	-	-	OMe
105	O-CH_2-O	-	-	-	-	OMe	-
106 (damnacznthol)	OMe	CH_2OH	OH	-	-	-	-
107	OH	CH_2OMe	OH	-	-	-	-
108 (juzunol)	OMe	CH_2OH	OH	-	OH	-	-
109	OH	CH_2Ome	OH	-	OH	-	-
110 (damnacanthal)	OMe	CHO	OH	-	-	-	-
111 (sennoside)13)							

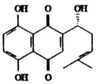


111

Figure 2.15 : Chemical structures of some naphtoquinones isolated from the plants of family Annonaceae



Compound	Substituents			
	C-2	C-5	C-6	C-8
112	-	-	-	-
113(juglone)	-	OH	-	-
114 (naphtazalin)	-	OH	-	OH
115	-	-	OH	-
116 (vitamin K_3)	Me	-	-	-
117	NMe_2	-	-	-
118	NMe_2	-	-	OH
119	NMe_2	OH	-	-
120	NMe_2	OH	-	OH
121	NMe_2	-	OH	-
122 (shikonin)				
123 (vitamin K_1)				
124				
125				
126				
127				



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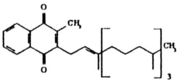

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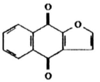

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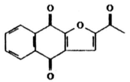

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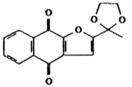
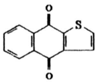
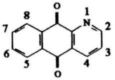


126

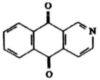


127

Figure 2.16 : Chemical structures of some azaanthraquinones isolated from the plants of family Annonaceae



Compound	Substituents					
	C-2	C-3	C-4	C-5	C-6	C-8
128 (cleistopholine)	-	-	Me	-	-	-
129	-	-	Me	-	-	OH
130	-	-	Me	OH	-	OH
131	-	-	Me	OMe	-	OMe
132	-	-	Me	-	OH	-
133	-	Me	-	-	-	-
134	-	Me	-	-	-	OH
135	-	Me	-	-	-	OMe
136	-	Me	-	OH	-	OH
137	Me	-	-	-	-	OH
138	Me	-	-	OH	-	OH
139						
140						
141						
142 (onychine)						
143						
144						



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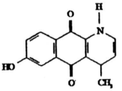

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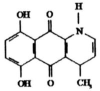

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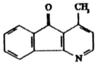

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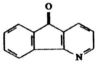

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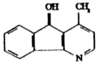


144

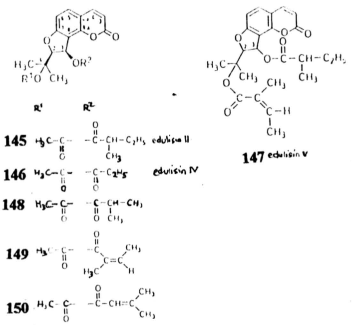
Coumarins

From the fruits of *Angelica endulis* Miyabe (Umbelliferae), angular-type furanocoumarins were isolated by Mizuno *et al.* (1994). They were endulisin III (145), endulisin IV (146), endulisin V (147), 2'(S), 3'(R)-3'-isobutyryloxy-4'-acetoxy-2', 3'-dihydrooroselol (148), endultin (149) and 2'(S), 3'(R)-3'-senecioyloxy-4'-acetoxy-2'-3'-dihydrooroselol (150). The structure of these compounds were shown in Figure 2.17.

This plant is widely distributed in the northern regions of Japan and was long utilized as an antiseptic by mixing it with fish oil and eating of its vegetable soup by the ancient Ainu people.

The inhibitory effect of these six furanocoumarins were examined on TPA-stimulated ^{32}Pi incorporation into phospholipids of HeLa cells. All coumarins showed potent inhibitory effects at a concentration of 50 μg/ml. Among these compounds, 147 showed 100% inhibition at 10 μg/ml. Whereas, the compounds having an acetoxyl group at C-4', 146 possesing apropyl group at C-3', 148 having an isobutyryl group at C-3', and 149 bearing an angeloyl group at C-3', showed higher inhibitory effects : 98.7%, 86.4% and 97.3% than 145. Coumarin 145 having a 2-methylbutyryl group at C-3' abd acetoxyl group at C-4' showed relatively less inhibitory action.

Figure 2.17 : Chemical structures of angular-type furanocoumarins isolated from the fruits of *A. endulis*



145 endulisin II

146 endulisin IV

147 endulisin V

148 2' (S), 3' (R-3'-isobutyryloxy-4'-acetoxy-2', 3'-dihydrooroselol

149 endultin

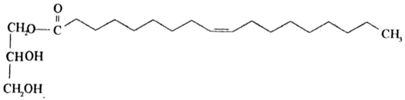
150 2' (S), 3' (R-3'-senecioyloxy-4'-acetoxy-2', 3'-dihydrooroselol

iv Crude Drugs

The seed of *Coix lachryma-jobi* L. var. *mayuen* Stapf (Graminae) represent one of the important Chinese herbal medicines used as a diuretic and anti-imflammatory drug, and also used in the treatment of pappilomas in Japanese medicine (Tokuda *et al.*, 1990). As part of their continuing studies, on biological activities of natural products, Tokuda *et al.*, (1990) have investigated the potential anti-tumour promoting activities of *Coix* seed. They have found that the methanolic extract of Coix seed exhibited a strong inhibitory effect on EBV EA activation with complete inhibition of plant extract concentration of 50 μg/ml.

Bioassay-directed fractionation of this extract led to the isolation of a monoglyceride, α-monolinolein (Figure 2.18). In the two stage *in* vivo carcinogenesis test using dimethylbenzaanthracene as an initiator and TPA as a promoter on ICR mice, both the methanol extract and α–monolinolein exhibited an anti-tumour promoting activity.

Figure 2.18 : Chemical structures of α-monolinolein isolated from the seed of *C. lachryma-joby* var. *mayuen*



α-monolinolein

2.5 Bioassays

According to Hamburger and Hostettmann (1991), the discovery of promising extracts and the subsequent activity-guided isolation put specific requirement on the bioassays to be used for that purpose. For compatibility with the large numbers of samples to be tested, the assays have to be simple, rapid reproducible and inexpensive. In the crude extract, active principles are generally present at low concentration only. The test system has, therefore, to be sensitive enough to detect them reliably. Its selectivity should be such that the number of false positive is reasonably small. In particular, the assay should ideally be insensitive to possible interferences from plants metabolites such as tannins, etc. Poor solubility of the extracts and fractions under test condition is quite common. False positive and false negative results are therefore, a much more serious problem than when dealing with pure compounds (Anderson *et al.*, 1991).

With a deeper understanding of cell biology and molecular pharmacology, mechanism-based bioassays have become increasingly important. Due to their selectivity and sensitivity combined with good reproducibility and high sample throughput, this type of assay is given preference for large screening programs in industry or in a collaborative setting (Suffness and Pezzuto, 1991).

2.5.1 Brine Shrimp Lethality Bioassay

In vivo lethality in a simple zoologic organism can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. The crustacen *Artemia salina* Leach. (brine shrimp) has been proposed as a low cost substitute for cytotoxicity assay (Meyer *et al.*, 1982). The assay has been mainly used by Professor J.L McLaughlin's group at Purdue University. It is based on the premise that bioactive compounds are toxic at higher doses and that lethality in a simple organism might be used as a mean of monitoring activity directed fractionation. The egg of brine shrimps will hatch within 48 hours upon being placed in sea water to provide a large number of larvae (nauplii) for experimental use.

2.5.2 Anti-Tumour Promoter Activity Bioassay

Okamoto *et al.* (1983) reported that using a short term system in which inhibition on TPA-induced EBV activation in Raji cells is very effective in search for possible anti-tumour promoters. Systematic studies with this test system were succeessful in purifying possible anti-tumour promoters from one of the Chinese medicinal plants, *Gleochoma hederaceae* L. (Labiate). This fact is supported by Nishino *et al.* (1984) who suggested that EBV EA inhibition assay using Raji cells was effective for the first screening of inhibitors of tumour promotion.

This assay system was first developed by Ito *et al.* (1981) for detecting tumour promoters utilizing the induction of the EA on EBV in lymphoblastoid Raji cells which carried the EBV genome. The assay system is rapid and efficient for detecting EBV-active principles in the environment. It could also detect tumour promoters at the ng/ml level and the result were available in 48 hours. The assay can thus be used as a screening test in the search for tumour promoters substances in nature.

In a survey made to screen potent tumour promoters in the environment, TPA was isolated from croton oil obtained from the seed of *Croton tiglium* (Hecker *et al.*, 1969). Since TPA is the most potent tumour promoter among a series of phorbol esters isolated from croton oil, it has been widely used in research that involved screening for anti-tumour promoter activity. Interestingly also, Ito *et al.* (1981), noted that low concentration of sodium n-butyrate increased the effect of tumour promoter synergistically, while naturally occuring tumour promoters have powerful irritant effect on mouse skin (Fujiki *et al.*, 1979).