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Growing Milkweed
a plant with prospective anti-cancer properties

A review of the medicinal potential of members of the ***Euphorbiaceae*** family.

A report for the Rural Industries Research and Development Corporation

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Foreword

The economy of Australia since settlement has depended heavily on primary production. Continuing innovation in the development of products and technology has been required to cope with changing world markets and conditions. At the personal level, the harsh Australian environment has taken a toll on the health of rural stakeholders, as well as on the Australian population at large. This can be seen in the high incidence of skin cancer, particularly in outdoor workers.

The present project may result in a unique opportunity for primary producers to contribute to their own wellbeing through the development of an effective treatment for non-melanoma skin cancer. A new, specialist crop might also emerge.

Compounds from a common but tractable weed are being developed for this purpose by a Brisbane-based, Australian-owned biotechnology company, Peplin Biotech Pty Ltd. Complementary expertise is being contributed by the Queensland Department of Primary Industries and by the Queensland Institute of Medical Research, one of the biggest medical research institutes in Australia.

The aim is to evaluate methods for the complete mechanisation of milkweed cultivation. This will include methods of seed production. The requirements for large-scale agriculture will be ascertained, using the expertise and equipment pooled from the wide variety of specialists in the Queensland Department of Primary Industries. Economical agricultural production is a major requirement for progressing the skin cancer treatment.

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This report, a new addition to RIRDC's diverse range of over 700 research publications, forms part of our Essential Oils and Plant Extracts R&D program, which aims to support the growth of a profitable and sustainable essential oils and natural plant extracts industry in Australia.

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Peter Core
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Abbreviations

BCC	Basal cell carcinoma
DPI	Department of Primary Industries
EBV	Epstein Barr Virus
E.peplus	*Euphorbia peplus*
HPTLC	High Performance Thin Layer Chromatography
SCC	Squamous cell carcinoma
QIMR	Queensland Institute of Medical Research
UV	Ultraviolet

Contents

Executive Summary

Peplin Biotech has discovered a novel class of natural compounds, purified from *Euphorbia*, which show great potential in the laboratory as a potent treatment for a wide range of human cancers including breast and prostate cancer, leukaemia, melanoma and other skin cancers, as well as lung, colorectal and cervical cancer.

An early clinical trial on thick and thin non-melanoma skin cancers has confirmed that the compounds are very effective in producing long-term (possibly permanent) responses in human patients without any evident systemic toxicity when applied topically. This is highly significant because current topical methods of drug treatment require long periods of application without being fully effective, and physical methods (*e.g.* surgery) are expensive and difficult to apply to the large areas affected, especially in older people.

Topical treatment of non-melanoma skin cancer - basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) - will be the first medical application of the *E.peplus* product. These are the most common human cancers, and most Australians will develop one or more BCC or SCC in their lifetimes. Mortality from melanoma is about 50% in the state of Queensland and morbidity is high. The treatment of non-melanoma skin cancer costs over $200 million dollars in Australia, making it the most expensive cancer. The rate of increase in skin cancer has slowed, presumably due to education in prevention measures but the incidence continues to be high and may increase with the ageing of the population. Western countries with less UV flux than Australia also have high incidences of non-melanoma skin cancer, due to sun-seeking lifestyles. In the US, the annual cost of non-melanoma skin cancer has been estimated at US$3.5 billion. There is therefore a need for a simple, reliable method of treating skin cancer on a large scale.

Over 70% of current pharmaceuticals are natural products or have been developed from a natural product lead. *E.peplus* is one of a number of plants in the *Euphorbiaceae* family that has attracted attention as a home remedy for skin cancer because of its milky sap. However, a survey by Peplin Biotech of over 200 species of the *Euphorbiaceae* family has shown that only *E.peplus* has the desired attributes of anti-cancer efficacy *in vitro* and *in vivo*. In addition, *E.peplus* is not a noxious weed, it grows rapidly, produces harvestable seed and it is potentially suitable for large-scale agricultural production. Thought to originate from Europe, *E.peplus* is now widespread throughout the world and can be found in most gardens.

Methods have been developed by Peplin Biotech for the extraction and purification of compounds from *E.peplus* in the laboratory, and these are currently being scaled-up for commercial production. It is anticipated that the overall cost of producing the active ingredient in pure form will result in a commercially viable treatment, provided the cost of agricultural production can be kept low.

The limiting factor in capturing commercial value from these discoveries is therefore the availability of plant feedstock for extraction of the active compounds. Developing large-scale agricultural production can solve this problem. Currently, the plant is sown by hand and tended in plots of 10-100 sq m. Harvesting of seed and plant is also carried out by hand. Sufficient information has now been obtained to show that there is no inherent difficulty in large-scale production in a variety of locations and environments in Australia.

We plan to evaluate methods for complete mechanisation of *E.peplus* cultivation and sap production. This will include methods of seed production, since the current supply of seed will need to be greatly expanded. The requirements for large-scale agriculture will be ascertained, using the expertise and equipment pooled from the wide variety of specialists in the DPI. The next critical step is to develop methods suitable for broad-acre, mechanised production.

Background on members of the *Euphorbiaceae* family

The *Euphorbiaceae* family covers a wide variety of plants (>2000 world-wide) including weeds, trees and other types of plants of the *Euphorbia* species, many of which are slow-growing succulents (http://florawww.eeb.uconn.edu/acc_num/198500271.html). When the stems are cut, many members exude a milky sap (latex) which has an unpleasant taste and is toxic when ingested in significant quantities. Such chemical properties, combined with the presence of thorns on some species (*e.g.* Crown of Thorns), presumably protect the plant from grazing in the arid regions of the world where many of the family originate.

A variety of inconclusive reports on the therapeutic potential effects of the sap of these plants have been published along with reports of tumour promotion and skin and ocular irritation.

The most intensively studied species of this group is *Euphorbia pilulifera* L (synonyms *E.hirta* L., *E.capitata* Lam.), whose common names include pill-bearing spurge, snakeweed, cat's hair, Queensland asthma weed and flowery-headed spurge. The plant is widely distributed in tropical countries, including India, and in Northern Australia (including Queensland).

A recent report describes selective cytotoxicity of a number of tiglilane diterpene esters from the latex of *Euphorbia poisonii*, a highly toxic plant found in Northern Nigeria, which is used as a garden pesticide. One of these compounds has been reported to have a selective cytotoxicity for the human kidney carcinoma cell line A-498 more than 10,000 times greater than that of Adriamycin (Fatope *et al.*, 1996).

Euphorbia hirta plants and extracts thereof have been considered for a variety of purposes, including tumor therapy (European Patent Application No. 0 330 094), AIDS-related complex and AIDS (Hungarian Patent Application No. 208790) and immune stimulation and anti-fungal treatment of open wounds (German Patent Application No. 4102054).

There are isolated reports of anti-cancer activity of various *Euphorbia* preparations (see Fatope *et al.*, 1996; Oksuz *et al.*, 1996; http://www.floridaplants.com/Med/cancer.htm). On the other hand, compounds present in at least one *Euphorbia* species were reported to be carcinogenic (Evans and Osman, 1974; Stavric and Stoltz, 1976; Hecker, 1971), one species has a skin-irritant and tumour-promoting effect (Gundidza and Kufa, 1993) and another species reduces EBV-specific cellular immunity in patients with Burkitt's lymphoma (Imai, 1994).

Given the above diversity of bioactivity amongst members of the *Euphorbiaceae* family, it is notable that Peplin Biotech has identified compounds from *E.peplus* (of the *Euphorbiaceae* family) which are effective for the treatment of skin cancer in humans.

Community use of *Euphorbia peplus*

Euphorbia peplus, commonly known in Australia as the radium weed or milkweed, is a non-invasive weed, most probably introduced to Australia by European settlers in the early 1800s for its medicinal value (Maiden, 1917; Pearn, 1987). Early Australian folklore suggested that the irritant sap had application against warts, corns, waxy growths, sun cancer and rodent ulcers (Maiden 1917; Hartwell, 1969). In Europe, folklore included the treatment of asthma and catarrh (Rizk *et al.*, 1985; http://www.homeoint.org/clarke/e/euphorb_peplus.htm), and as a purgative. *E.peplus* was widely used in the Ukraine around the turn of the century as a treatment for cancer of the stomach, liver and uterus (Shimansk'ka, 1961). A letter to the Medical Journal of Australia reported a case of self-treatment using *E.peplus* sap to remove a basal cell carcinoma from the chest by a local farmer (Weedon and Chick, 1976). More recently, a survey of home remedies for skin cancer and solar keratoses used by residents of the Nambour district of Queensland concluded that *E.peplus* was unanimously considered an effective treatment (Green and Beardmore, 1988).

Development of anti-cancer compounds from *Euphorbias* by Peplin Biotech Pty Ltd

Peplin Biotech Limited is a Brisbane-based Life Sciences Company which is listed on the Australian Stock Exchange. Its present sole activity is research and development to support the commercialisation of a novel group of compounds discovered in 1997 by its founder, Dr James Aylward, a former CSIRO biochemist. It conducts all of its research by way of external contracts with research organisations such as the Queensland Department of Primary Industries, the Queensland Institute for Medical Research, the CSIRO, as well as at various universities and hospitals. A more complete description of the Company and its undertakings can be found at http://www.peplin.com.

The Queensland Department of Primary Industries (DPI) is currently playing a key role in optimising the conditions for agriculture of *E.peplus*, a plant which, although ubiquitous as a weed in gardens and nurseries, has never before been cultivated. Covering possibly the widest range of climates in Australia, the DPI has a broad range of expertise in all aspects of agriculture, including the development of exotic crops. Current small-scale experimental work with *E.peplus* involves the DPI's Centre for Food Technology and Queensland Horticulture Institute establishment at Redlands, near Brisbane.

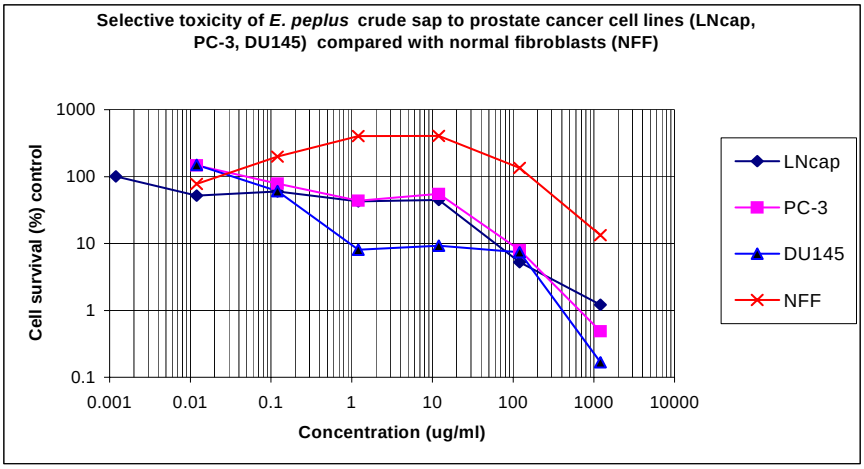
The Queensland Institute for Medical Research (QIMR) is one of the largest biomedical research institutes in Australia, with a staff of approximately 400. A significant proportion of its effort is devoted to cancer research, and expansion into a new Comprehensive Cancer Centre is currently underway. The contribution of QIMR in the present context is to assay the compounds produced by *E.peplus* under various conditions of agriculture, using chemical and bioactivity measurements.

The means are available to conduct clinical trials with a purified compound, delivered in a suitable cream. Problems of extraction of compound and purification have been largely overcome. The rate-limiting step at present is shortage of compound, due to lack of knowledge about large-scale agriculture.

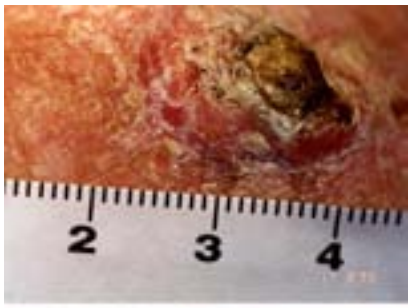
We plan to evaluate methods for complete mechanisation of *E.peplus* cultivation and sap production. The requirements for large-scale agriculture will be ascertained, using the expertise and equipment pooled from the wide variety of specialists in the DPI. The next critical step is to develop methods suitable for broad-acre, mechanised production.

A clinical trial with *E.peplus* latex in Brisbane

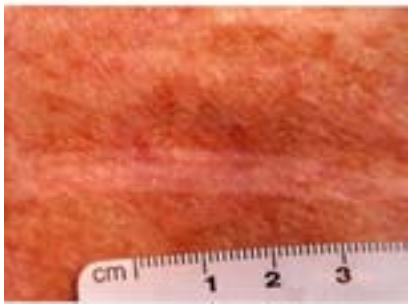
With the above background, Peplin Biotech began a study to determine the performance of crude sap against a variety of cancers, with an emphasis on skin cancers. Surprisingly, the sap was not only efficacious against a variety of skin cancer cell lines, including strains of malignant melanoma resistant to conventional chemotherapeutic agents, but also had powerful cell inhibitory activity against a wide range of other tumours tested, including breast cancer cells.



Skin cancer



12 weeks after 3 daily treatments



This promising *in vitro* data, coupled with the extensive community use for the topical treatment of skin cancer without any reported side effects, resulted in ethics approval being granted for a Phase I/II clinical trial of the crude sap on skin cancer patients at the Mater Hospital in Brisbane. The trial was restricted to patients who had failed or refused conventional therapy. The skin cancers treated in the trial, therefore, tended to be at an advanced stage and some were on sites where healing after surgery or radiotherapy was a serious problem (*e.g.* the lower leg in older patients). Long-term complete remissions were confirmed by biopsy and were >80%.

Active compounds from *E.peplus*:

Structure and bioactivity assays

One of the first observations made in current research on *E.peplus* sap was that it was selectively toxic to tumour cells compared with normal cells in culture. Moreover, at concentrations of the sap too low to effect direct cell killing, surviving tumour cells examined by light microscopy demonstrated an altered morphology (*i.e.* a dendritic appearance). In the case of melanoma, the morphological changes were characteristic of normal melanocytes. Further cell observations led to the conclusion that the diluted sap was acting as a potent differentiation control agent.

Bioactivity guided fractionation has led to the identification of the active principles responsible for the selective cytotoxicity and differentiation control activity. The activity resided in macrocyclic diterpenes of three families, namely ingenane, pepluane and jatrophane. Pepluane and jatrophane are novel molecules with unusual conformations. First documented in the literature in 1998 by Jakupovic *et al.*, they are reported to be non-inflammatory. Ingenane, on the other hand is responsible for the irritant properties of the latex (Jakupovic *et al.*, 1998). HPTLC chromatographic techniques were developed at QIMR to enable the separation and concentration of sufficient quantities of each of these components to undertake further *in vitro* and *in vivo* studies. The specific molecules in question are: 20-acetyl-ingenol-3-angelate (ingenane) and other ingenol esters - 5,8,9,10,14-pentaacetoxy-3-benzoyloxy-15-hydroxypepluane (pepluane), 15-pentaacetoxy-9-nicotinoyloxy-14-oxojatropha-6 (1),11E-diene (jatrophane 1), 2,5,7,9,14-hexaacetoxy-3-benzoyloxy-15-hydroxy-jatropha-6(17),11*E*-diene (jatrophane 2), 2,5,14-triacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutyroyloxy-9-nicotinoyloxyjatropha-6(17),11*E*-diene (jatrophane 3), 2,5,9,14-tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutyroyloxyjatropha-6(17),11*E*-diene) (jatrophane 4), 2,5,7,14-tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-9-nicotinoyloxyjatropha-6(17),11E-diene (jatrophane 5) and 2,5,7,9,14-pentaacetoxy-3-benzoyloxy-8,15-dihydroxyjatropha-6(17),11E-diene (jatrophane 6).

Mouse studies using *in vivo* models for skin cancer have been encouraging, with cures obtained when crude sap or purified diterpenes from *E.peplus* were used topically on human malignant melanoma xenografts in nude mice and B16 mouse melanoma in C57 black mice.

Intellectual property surrounding the use of ingenane, jatrophanes and pepluane as anti-neoplastic differentiation control agents are the subject of patent application No. PCT/AU98/00656 and are the property of Peplin Biotech Pty Ltd.

Novel mechanism of anti-cancer action

Key results have been obtained from a range of tumour and normal cells (primarily the human melanoma cell line MM96L), indicating that a particular enzyme (a kinase) is the primary target for the *E.peplus*-derived compounds. This results in a signal cascade inside the cell which affects growth, survival, differentiation and release of chemokines which communicate to other cells and tissues. The overall outcome for cancer control probably results from a combination of many of these changes.

As part of ongoing drug development by Peplin Biotech, a range of assays have been established to compare the bioactivities of crude extracts from plants as well as pure compounds. We are, therefore, in a strong position to rapidly evaluate any changes in bioactivity.

Screening of other members of the family for bioactivity

The *Euphorbiaceae* plant family consists of over 2000 members but only a few species are worth considering as commercial sources of compounds. This is based on our recent survey of >200 species made available through the Succulent Society of Queensland and the Queensland Herbarium. Most of these had significant *in vitro* activity but were succulents, which grow very slowly. Two other candidate species that have been cultivated (*Synadenium grantii* and *E.tirucalli*) are readily propagated and produce copious amounts of sap with high *in vitro* activity but their active diterpenes have been reported to be phorbol derivatives which may be less attractive for commercial development than the *E.peplus*-derived compounds. Several other weeds from the *Euphorbiaceae* family have been considered (see below).

Is *E.peplus* the most suitable species for production of anti-cancer compounds?

Euphorbia peplus grows wild in temperate climates around the world (http://www.nhm.ac.uk/science/projects/fff/ChekLisE.htm). While a number of other related species with milky saps (http://www.wa.gov/agr/weedboard/weed_info/leafyspurge2.html) have been considered, these have a range of drawbacks. *E.terracina*, *E.lathryis* and *E.davidii* grow in Australia as weeds but are listed as noxious weeds in various States and are therefore unacceptable for agriculture. In addition, our testing of *E.terracina* sap revealed insufficient bioactivity in culture but to cure tumours in the mouse model. *E.paralias* is a woody plant that grows on beach sand dunes along the southeastern coast of Australia. It is considered to be a noxious weed, both here and in Europe. The bioactivity of its sap is not high, the cultivatable areas are very limited and the plant grows slowly. *E.esula* is a seriously noxious weed in the USA and is not found in Australia. The *Euphorbia* succulents grow extremely slowly and would not produce enough biomass to be economically feasible sources of the desired compounds.

We have propagated some 1000 plants of the shrub *Synadenium grantii* but its sap has poor activity in the mouse model and contains a phorbol ester rather than the preferred ingenane esters. Similar results have been obtained with sap from the shrub *E.tirucalli* (naked lady).

E.peplus, therefore, remains our species of choice because of its ease of cultivation, history of safe community use and demonstrated efficacy in a clinical trial of the crude sap and in animal models using the purified compounds.

Agriculture of *E.peplus*

The large number of asymmetric centres in these molecules makes synthesis unattractive on a commercial scale. Attention has therefore been given to developing the plant source (see Appendix II for details of a student project). This work is currently progressing through agronomic trials undertaken with the Queensland Department of Primary Industries.

E.peplus grows readily from seed in temperate and subtropical climates, maturing in 12-14 weeks. We have grown it all the year round at appropriate locations in Australia. Potential viral problems have been discussed (http://image.fs.uidaho.edu/vide/famly058.htm). Rust may be a problem in the maturing stages. The seeds are small (1700/gram) but have >70% germination rate. Although found as a weed in many gardens and nurseries, *E.peplus* never spreads spontaneously beyond the limits of shade and is not listed as a noxious weed in any Australian State. It is easy to weed by hand, and is killed readily by weedicides including Roundup. It is therefore unlikely to become a serious competitor with other crops.

A process has been developed for the laboratory extraction and purification of compounds from green or dried plant material. Quality control of batches can be done quickly and inexpensively by *in vitro* bioassay and mass spectroscopy. Yield of plant is currently 1-1.5 kg/sq meter, and yield of compounds is approximately 100-300 mg/kg wet weight of plant.

With the assistance of the Queensland Department of Primary Industry, we plan to evaluate methods for complete mechanisation of *E.peplus* cultivation and sap production. It should be straightforward to determine the requirements for large-scale agriculture, given the expertise and equipment pooled from the wide variety of specialists in the DPI. The next critical step is to develop methods suitable for broad-acre, mechanised production.

References

Agus DB, Scher HI, Higgins B, Fox WD, Heller G, Fazzari M, Cordon-Cardo C and Golde DW. (1999) Response of prostate cancer to anti-Her-2/neu antibody in androgen-dependent and -independent human xenograft models. Cancer Res. 59(19):4761-4.

Anidjar M, Villette JM, Devauchelle P, Delisle F, Cotard JP, Billotey C, Cochand-Priollet B, Copin H, Barnoux M, Triballeau S, Rain JD, Fiet J, Teillac P, Berthon P and Cussenot O. (2001) *In vivo* model mimicking natural history of dog prostate cancer using DPC-1, a new canine prostate carcinoma cell line. Prostate 46(1):2-10.

Antalis TM, La Linn M, Donnan K, Mateo L, Gardner J, Dickinson JL, Buttigieg K and Suhrbier A. (1998) The serine proteinase inhibitor (serpin) plasminogen activation inhibitor type 2 protects against viral cytopathic effects by constitutive interferon alpha/beta priming. J. Exp. Med. 187(11):1799-811.

Bowtell DD. (1999) Options available - from start to finish - for obtaining expression data by microarray. Nature Genet. 21(1 Suppl):25-32.

Bushnell DL, Madsen M, Kahn D, Nathan M and Williams RD. (1999) Enhanced uptake of 99Tcm-MDP in skeletal metastases from prostate cancer following initiation of hormone treatment: Potential for increasing delivery of therapeutic agents. Nucl. Med. Commun. 20(10):875-81.

El Etreby MF, Liang Y, Johnson MH and Lewis RW. (2000) Antitumor activity of mifepristone in the human LNCaP, LNCaP-C4, and LNCaP-C4-2 prostate cancer models in nude mice. Prostate 42(2):99-106.

Elliott SL, Pye S, Le T, Mateo L, Cox J, Macdonald L, Scalzo AA, Forbes CA and Suhrbier A. (1999) Peptide based cytotoxic T-cell vaccines; delivery of multiple epitopes, help, memory and problems. Vaccine 17(15-16):2009-19.

Evans IA and Osman MA. (1974) Carcinogenicity of bracken and shikimic acid. Nature. 250(464):348-9.

Fatope MO, Zeng L, Ohayaga JE, Shi G and McLaughlin JL. (1996) Selectively cytotoxic diterpenes from *Euphorbia poisonii*. J. Med. Chem. 39(4):1005-8.

Gabizon AA. (2001) Pegylated liposomal doxorubicin: Metamorphosis of an old drug into a new form of chemotherapy. Cancer Invest. 19(4):424-36.

Gonzalez R, Ebbinghaus S, Henthorn TK, Miller D and Kraft AS. (1999) Treatment of patients with metastatic melanoma with bryostatin-1: A phase II study. Melanoma Res. 9(6):599-606.

Greaves MW and Wall PD. (1996) Pathophysiology of itching. Lancet 348(9032):938-40.

Green AC and Beardmore GL. (1988) Home treatment of skin cancer and solar keratoses. Australas J. Dermatol. 29(3):127-30.

Gundidza M and Kufa A. (1993) Skin irritant and tumour promoting extract from the latex of *Euphorbia bougheii*. Cent. Afr. J. Med. 39(3):56-60.

Han ZT, Zhu XX, Yang RY, Sun JZ, Tian GF, Liu XJ, Cao GS, Newmark HL, Conney AH and Chang RL. (1998) Effect of intravenous infusions of 12-O-tetradecanoylphorbol-13-acetate (TPA) in

patients with myelocytic leukemia: Preliminary studies on therapeutic efficacy and toxicity. Proc. Natl. Acad. Sci. USA 95(9):5357-61.

Hartwell JL. (1969) Plants used against cancer. A survey. Lloydia 32(3):247-96.

Hecker E. “Cocarcinogens from *Euphorbiaceae* and *Thymeleaceae”*. In: “Symposium on Pharmacognosy and Phytochemistry”. Pp 147-165. Eds. Horhammer L and Wagner H. Springer Verlag, NY. 1971.

Hoffman RM. (1999) Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic. Invest New Drugs 17(4):343-59.

Horsmanheimo L, Harvima IT, Harvima RJ, Brummer-Korvenkontio H, Francois G and Reunala T. (1996) Histamine and leukotriene C4 release in cutaneous mosquito-bite reactions. J. Allergy Clin. Immunol. 98(2):408-11.

Imai S, Sugiura M, Mizuno F, Ohigashi H, Koshimizu K, Chiba S and Osato T. (1994) African Burkitt's lymphoma: A plant, *Euphorbia tirucalli*, reduces Epstein-Barr virus-specific cellular immunity. Anticancer Res. 14(3A):933-6.

Jain R, Shah NH, Malick AW and Rhodes CT. (1998) Controlled drug delivery by biodegradable poly(ester) devices: different preparative approaches. Drug Dev. Ind. Pharm. 24(8):703-27.

Jakupovic J, Morgensternb T, Bittnera M and Silva M. (1998) Diterpenes from Euphorbia peplus. Phytochemistry 47(8):1601-1609.

Kanellakopoulou K and Giamarellos-Bourboulis EJ. (2000) Carrier systems for the local delivery of antibiotics in bone infections. Drugs 59(6):1223-32.

Klagsbrun M, Sasse J, Sullivan R and Smith JA. (1986) Human tumor cells synthesize an endothelial cell growth factor that is structurally related to basic fibroblast growth factor. Proc. Natl. Acad. Sci. USA 83(8):2448-52.

Kondoh T, Nakamura M, Nabika T, Yoshimura Y and Tanigawa Y. (1999) Ubiquitin-like polypeptide inhibits the proliferative response of T cells *in vivo*. Immunobiology 200(1):140-9.

Kubota T, Miyauchi M, Miura K, Hirokawa G, Awaya A, Miyasaka N, Kurosawa Y, Kanai Y and Maruyama K. (1998) Upregulation of nucleobindin expression in human-activated lymphocytes and non-Hodgkin's lymphoma. Pathol. Int. 48(1):22-8.

Kunisawa J and Mayumi T. (2000) Application of novel drug delivery system, fusogenic liposome, for cancer therapy. Gan To Kagaku Ryoho 28(5):577-83.

Linn ML, Aaskov JG and Suhrbier A. (1996) Antibody-dependent enhancement and persistence in macrophages of an arbovirus associated with arthritis. J. Gen. Virol. 77(3):407-11.

Maeda H, Segawa T, Kamoto T, Yoshida H, Kakizuka A, Ogawa O and Kakehi Y. (2000) Rapid detection of candidate metastatic foci in the orthotopic inoculation model of androgen-sensitive prostate cancer cells introduced with green fluorescent protein. Prostate 45(4):335-40.

Maiden JH. (1917) Weeds of New South Wales. Ag. Gazette NSW 28:131-132.

Marks R, Staples M and Giles GG. (1993) Trends in non-melanocytic skin cancer treated in Australia: The second national survey. Int. J. Cancer 53(4):585-90.

Matsushita S. (2000) Current status and future issues in the treatment of HIV-1 infection. Int. J. Hematol. 72(1):20-7.

Miller DL and Weinstock MA. (1994) Non-melanoma skin cancer in the United States: incidence. J. Am. Acad. Dermatol. 30(5): 774-778.

Mollinedo F, Borregaard N and Boxer LA. (1999) Novel trends in neutrophil structure, function and development. Immunol. Today 20(12):535-7.

Muggia FM. (2001) Liposomal encapsulated anthracyclines: New therapeutic horizons. Curr. Oncol. Rep. 3(2):156-62.

Murali-Krishna K, Altman JD, Suresh M, Sourdive DJ, Zajac AJ, Miller JD, Slansky J, Ahmed R. (1998) Counting antigen-specific CD8 T cells: A re-evaluation of bystander activation during viral infection. Immunity 8(2):177-87.

Navone NM, Logothetis CJ, von Eschenbach AC and Troncoso P. (1998-99) Model systems of prostate cancer: Uses and limitations. Cancer Metastasis Rev. 17(4):361-71.

Nishioka Y and Yoshino H. (2001) Lymphatic targeting with nanoparticulate system. Adv. Drug Deliv. Rev. 47(1):55-64.

Norris S, Halkar R, Galt J, Petros J and Keller J. (1999) Bone metastases from poorly differentiated adenocarcinoma of the prostate diagnosed by In-111 ProstaScint (Capromab Pendetide) images with negative results of Tc-99m MDP bone scan and without significant elevation of prostate-specific antigen. Clin. Nucl. Med. 24(11):905-7.

Oksuz S, Gurek F, Lin LZ, Gil RR, Pezzuto JM and Cordell GA. (1996) Aleppicatines A and B from *Euphorbia aleppica*. Phytochemistry 42(2):473-8.

Pearn J. (1987) The enchanted herb: The work of early medical botanists in Australia. Med. J. Aust. 147(11-12):568-72.

Piccart-Gebhart M. (1999) What can be expected from the next generation of adjuvant breast cancer treatment? Semin. Oncol. 26(1 Suppl 3):22-5.

Post PN, Straatman H, Kiemeney LA and Coebergh JW. (1999) Increased risk of fatal prostate cancer may explain the rise in mortality in The Netherlands. Int. J. Epidemiol. 28(3):403-8.

Rizk, A.M. Hammouda FM, El-Missiry MM, Radwan HM and Evans FJ. (1985) Biologically active diterpenes from *Euphorbia peplus*. Phytochemistry 24(7):1605-1606.

Schroder FH, Kranse R, Barbet N, Hop WC, Kandra A and Lassus M. (2000) Prostate-specific antigen: A surrogate endpoint for screening new agents against prostate cancer? Prostate 42:107-115.

Shimansk'ka, V.O. (1961) Use of certain plants for treatment of (exophthalmic) goiter and "cancer" in natural medicine. Nauk. Zap. Kremenets'k. dersh. Ped. Inst. 6:55-58.

Stavric B and Stoltz DR. (1976) Shikimic acid. Food Cosmet. Toxicol. 14(2):141-5.

Steinkamp JA, Wilson JS, Saunders GC and Stewart CC. (1982) Phagocytosis: Flow cytometric quantitation with fluorescent microspheres. Science 215(4528):64-6.
Thomasin C, Ho NT, Merkle HP and Gander B. (1998) Drug microencapsulation by PLA/PLGA coacervation in the light of thermodynamics. 1. Overview and theoretical considerations. J. Pharm. Sci. 87(3):259-68.

Tobiume M, Fujinaga K, Kameoka M, Kimura T, Nakaya T, Yamada T and Ikuta K. (1998) Dependence on host cell cycle for activation of human immunodeficiency virus type 1 gene expression from latency. J. Gen. Virol. 79(6):1363-71.

Vollmer RT and Montana GS. (1999) Predicting tumor failure in prostate carcinoma after definitive radiation therapy: limitations of models based on prostate-specific antigen, clinical stage and Gleason score. Clin. Cancer Res. 5(9):2476-84.

Weedon D and Chick J. (1976) Home treatment of basal cell carcinoma. Med. J. Aust. 1(24):928.

Wu TS, Lin YM, Haruna M, Pan DJ, Shingu T, Chen YP, Hsu HY, Nakano T and Lee KH. (1991) Antitumor Agents, 119. Kansuiphorins A and B, two novel antileukemic diterpene esters from *Euphorbia kansui*. J. Nat. Prod. 54(3):823-9.