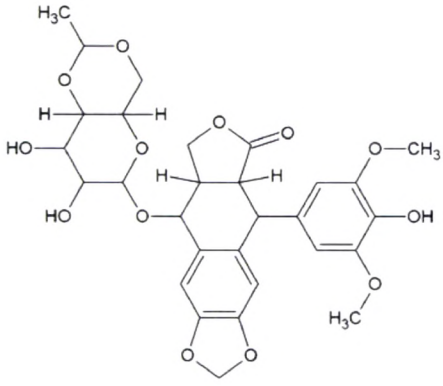
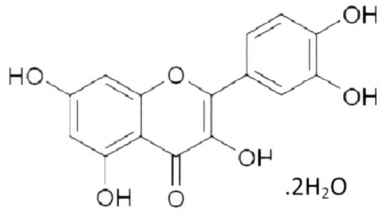
Chapter 3



ETOPOSIDE



QUERCETIN DIHYDRATE

DRUG PROFILES

3. DRUG PROFILES

3.1 ETOPOSIDE

3.1.1 Introduction:

Etoposide is a semisynthetic podophyllotoxin-derivative antineoplastic agent. Etoposide differs structurally from podophyllotoxin by having a glucoside moiety. It is cytotoxic agent used in the treatment of malignant disease, typically as a part of combination therapy with other chemotherapeutic agents.

3.1.2 Description:

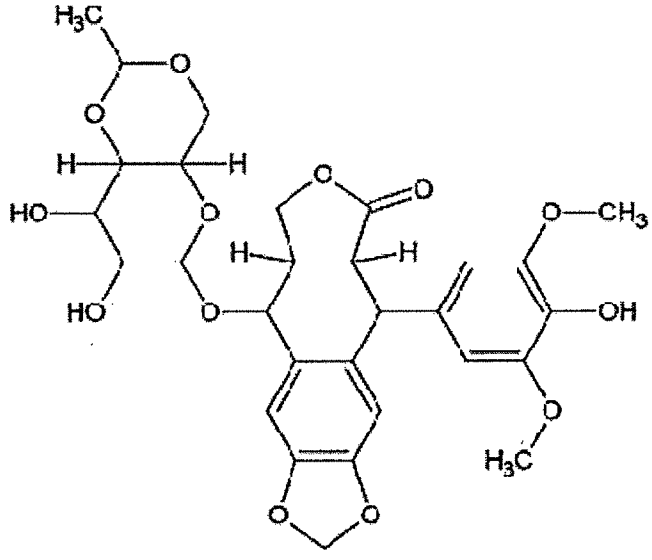
3.1.2a Chemical name:

9-(4,6-O-ethylidene-β-D-glucopyranosyloxy)-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-isobenzofuro[5,6-f][1,3]benzodioxol-6(5aH)-one.

4'-Demethylepipodophyllotoxin 9-(4, 6-O-Ethylidene-β-D-Glucopyranoside) (Sigma MSDS)

3.1.2b Empirical formula: $C_{29}H_{32}O_{13}$

3.1.2c Structural formula:



3.1.2d Molecular weight: 588.6

3.1.2e Appearance and colour:

White to white with a yellow cast crystalline powder.

3.1.3 Physicochemical properties:

3.1.3a Solubility:

Etoposide is sparingly soluble in water (approximately 0.03 mg/mL) but soluble in organic solvents such as ethanol, methanol and DMSO to different extents (approximately 0.76 mg/mL in alcohol); the water miscibility of the drug is increased by the presence of organic solvents. (Martindale, 1982)

3.1.3b Melting point: 236-251°C

3.1.3c Dissociation constant:

The pKa of Etoposide is 9.8.

3.1.3d Partition coefficient:

The Octanol/Water partition coefficient of Etoposide is 9.94

3.1.4 Pharmacology:

***Mechanism of action*:**

It inhibits DNA topoisomerase II, thereby inhibiting DNA synthesis. Etoposide is cell cycle dependent and phase specific, affecting mainly the S and G_2 phases. Topoisomerase II is amulti-subunit enzyme which uses ATP to pass an intact helix through a transient double-stranded break in DNA to modulate DNA topology (Watt and Hickson,1994). After strand passage, the DNA backbone is religated and DNA structure restored. Etoposide prevents topoisomerase II from religating cleaved DNA (Fortune and Osheroff, 2000). Etoposide thus converts topoisomerase II into a poison that introduces high levels of transient protein-associated breaks in the genome of treated cells.

Topoisomerase II exists as two highly homologous isoforms,alpha and beta, which differ in their production during the cell cycle. The alpha isoform concentration increases 2–3-fold during G2/M, and orders of magnitude is higher in rapidlyproliferating cells than in quiescent cell populations. The alpha isoform appears to be the target of etoposide (Gatto and Leo, 2003). Two scissile bonds are formed per every topoisomerase II-mediated double-stranded DNAbreak. Results of DNAcleavage and ligation assay studies indicate a two-site model for the action of etoposide against human topoisomerase II alpha. This model suggests that drug interactions at both scissile bonds are required in order to increase enzyme-mediated double-stranded DNA breaks (Bromberg KD 2003). Subsequent cell death is typically by apoptosis. The cell-signaling pathways that lead to apoptosis following topoisomerase-induced DNA damage are not completely understood.

Pharmacokinetics:

Etoposide is poorly soluble in water. For intravenous use, etoposide is dissolved in a solubilizer composed of polysorbate 80, polyethylene glycol, and alcohol and diluted to a concentration less than 0.4 mg/ml to avoid precipitation. Approximately one-third of intravenously administered etoposide is excreted in the urine. Less than 2% of an administered etoposide dose is excreted into bile as intact drug. Hepatic glucuronidation accounts for 25% of etoposide's clearance. Etoposide is also metabolized to a reactive catechol metabolite by cytochrome P450 3A4, which also has cytotoxic activity. Etoposide is highly bound to plasma proteins with only 6–8% being non-bound (Hande, 2003).

Following table presents the pharmacokinetics of Etoposide in detail.

Table 3.1 Pharmacokinetics of Etoposide (Adapted from BC Cancer Agency, Cancer Drug Manual, 2006)

Interpatient variability	Bioavailability	
Oral Absorption	dose-dependent; absorption decreases as etoposide dose increases; mean 50%. daily doses greater than 200 mg should be divided (BID) absorption does not appear to be altered by food or changes in stomach pH and emptying; however manufacturer recommends drug be taken on an empty stomach.	
	time to peak plasma conc.	1-1.5 h
Distribution	detected in saliva, liver, spleen, kidney, myometrium,healthy brain tissue, and brain tumor tissue, minimally in pleural fluid	
	cross blood brain barrier?	in low and variable concentrations
	volume of distribution	7-17 L/m^2, 32% of body weight
	plasma protein binding	95%
Metabolism	hepatic biotransformation	
	active metabolite	yes
	inactive metabolite	yes
Excretion	fecal and urinary excretion	
	urine	44-60% (67% of that unchanged)
	faeces	up to 16% (as unchanged drug and metabolites)
	biliary	≤6%
	terminal half life	7 h (range, 3-12)
	clearance	19-28 mL/min/m^2
Gender	no clinically important differences	
Elderly	no clinically important differences	
Children	volume of distribution 5-10 L/m^2,terminal half life 3-5.8 h	

Drug interactions:

Only a few drug interactions have been identified that involve etoposide. Concomitant use of prednisone induces etoposide clearance, possibly through induction of P-glycoprotein. Patients receiving glucocorticoids may be relatively under dosed as induction of P-glycoprotein may increase renal or biliary clearance. Inhibitors of P-glycoprotein delay etoposide clearance, increasing toxicity.

Indications and dosage:

Etoposide is reportedly used in the treatment of different malignant neoplasms such as leukemia, and tumors of the brain, lung, testis and stomach (Martindale, 1982). Specific indications include Non-Hodgkin's lymphoma, Hodgkin's disease, non-small cell lung carcinoma, acute leukemias, small cell carcinoma of bronchus, non-seminomatous testicular cancer, neuroblastoma, Aids related Kaposi's sarcoma and gastric cancer.

Doses of 120mg/m^2 to 240mg/m^2 of etoposide by intravenous administration daily for 5 days. Oral dose is twice the intravenous dose.

Toxicology:

Common toxicities from etoposide include bone marrow suppression, nausea, vomiting, and alopecia. At very high doses, such as those used with bone marrow transplantation regimens, mucositis becomes the dose-limiting toxicity. Liver toxicity, fever, and chills may also occur with highdose therapy. Palmar-plantar eruptions and irritation of the anal canal have been associated with etoposide use. Hypersensitivity reactions, including vasomotor changes in the pulmonary and gastrointestinal systems, may also occur following etoposide use. These reactions may result from the Tween 80 needed to solubilize etoposide. The most serious adverse event associated with etoposide is the development of acute myelogenous leukemia (Hande, 2003). On the basis of current clinical evidence, the World Health Organization has identified etoposide as carcinogenic to humans (Anonymous, 2000).

3.1.5 Analytical methods:

There are number of methods available for estimation of etoposide in various working solutions and biological fluids. Some of them are listed below.

- Etoposide exhibits UV absorption maxima in ethanol and absolute methanol at 283nm with molar absorptivity of 4245.
- Jasti et al. (2003) reported HPLC method for etoposide estimation using C_8 reversed phase column and acetonitrile-acetic acid-water (34:1:65) as mobile phase at pH 4.0. The flow rate was 1.5 ml/min, and the eluents were monitored at 230 nm.

- Reddy et al. (2006) reported estimation of etoposide by UV spectroscopy using 1:1 methanol : chloroform solvent system at 286nm.
- Chu et al. (2003) reported HPLC method for etoposide estimation in plasma using aqueous 10% methanol at 228nm.
- Hamied and co-workers (1991) reported a simple spectrophotometric method, wherein the samples were diluted with methanol and treated with 1% potassium periodate. After dilution with methanol, the absorbance of the solution was measured at 290nm vs. a reagent blank.

3.2 QUERCETIN DIHYDRATE

3.2.1 Introduction:

Quercetin Dihydrate is a dihydrate form of Quercetin. Quercetin is polyphenolic bioflavonoid, more specifically flavonol. Quercetin is found in many fruits and vegetables such as broccoli, lettuce, apples, tomatoes, onions, as well as in olive oil, red wine, tea, and coffee. (Boik, 2001; Spoerke, 2003) Much of the recent research on Quercetin has shown that it is a potential anticancer compound. It possesses many functions including antioxidation, blocks cellular signal transduction inhibits the ras cascade, which is important for cellular proliferation and induces apoptosis (Boik, 2001; Spoerke, 2003). Quercetin also showed significant antiangiogenic activity by inhibiting several important steps in angiogenesis process (Igura et al. 2001; Wen-fu tan et al. 2003)

3.2.2 Description:

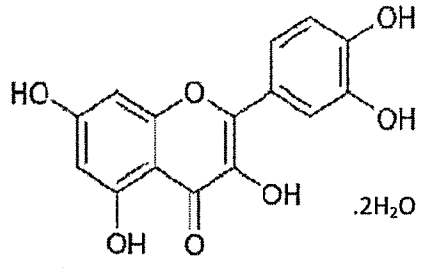
3.2.2a Chemical name:

2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one dihydrate.

3,3',4',5,7-penthydroxyflavone dihydrate.

3.2.2b Empirical formula: $C_{15}H_{10}O_7.2H_2O$

3.2.2c Structural formula:



3.2.2d Molecular weight: 338.27

3.2.2e Appearance and colour:

Bright yellow to greenish yellow coloured powder.

3.2.3 Physicochemical properties:

3.2.3a Solubility:

Very slightly soluble in hot water, diethyl ether, insoluble in cold water, soluble in ethanol and acetone (Lide, 1997) , soluble in acetic acid, pyrimidine and in DMSO (24 mg/ml)

3.2.3b Melting point: >300°C (572°F)

3.2.3cExtinction Coefficient:

E^{mM} = 19.95 (257 nm, 95% ethanol) and 21.88 (376 nm, 95% ethanol) (Merck Index).

3.1.4 Pharmacology:

Quercetin appears to have many beneficial effects on human health, including cardiovascular protection, anti-cancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity, and anti-inflammatory effects but the present discussion is limited to effects related to anticancer and antiangiogenic activities.

Mechanisms of action

Quercetin Dihydrate is a potential anticancer compound. It possesses many functions including antioxidation, inhibits cell cycle at G1 and S phase in vitro, reversal of multidrug resistance, inhibits phosphorylation of protein kinase C (PKC) and tyrosine kinase, which ultimately blocks cellular signal transduction leading to decreased tumor growth. It also inhibits the ras cascade, which is important for cellular proliferation and induces apoptosis

(Boik, 2001; Manach et al., 1996), binds to type II estrogen receptors to stimulate growth inhibition/proliferation (Lamson and Brignall, 2000; Boik, 2001; Spoerke, 2003).

Quercetin inhibits the mutation of the tumor suppressor protein gene p53. The mutation, or defect of this suppressor is involved in more than half of all cancer cell lines including breast, ovarian and prostate cancers. Quercetin strongly inhibited, in a time- and dose dependent fashion, the expression of the mutated p53 protein, in breast cancer. (Avila et al., 1994).

Quercetin has been found to inhibit production of heat shock proteins in several malignant cell lines, including breast cancer, leukemia, and colon cancer. Heat shock proteins form a complex with mutant p53, which allows tumor cells to bypass normal mechanisms of cell cycle arrest. Heat shock proteinsalso allow for improved cancer cell survival under different bodily stresses (low circulation,fever, etc.) (Lamson and Brignall, 2000)

Quercetin also showed significant antiangiogenic activity. Quercetin inhibited several important steps of angiogenesis including proliferation, migration, and tube formation of endothelial cells in vitro and exerted antiangiogenic activity in vivo.

It has also shown to inhibit Matrix metalloproteinase-2 which plays important role in proteolytic degradation of the extracellular matrix that is required for migration and invasion of endothelial cells at the start of angiogenesis. (Igura et al., 2001, Wen-fu tan et al., 2003)

It has also shown to inhibit Tyrosine kinases which are a family of proteins located in or near the cell membrane involvedin the transduction of growth factor signals to the nucleus. (Lamson and Brignall, 2000)

Quercetin is found to be able to suppress the VEGF-stimulated HUVEC tubular structure formation and to inhibit the activated U937 monocytic cell adhesion to HUVEC cells, through playing an important role in the prevention of angiogenesis. (Kim J 2006)

It inhibits angiogenesis through multiple mechanisms. These include interaction with the COX-2 and lipoxygenase (LOX)–5 enzymes, the EGF receptor, the HER-2 intracellular signaling pathway, and the NF-κB nuclear transcription protein. A prostate cancer xenograft model showed that quercetin could enhance the anticancer effects of tamoxifen through antiangiogenesis. (Yance and Sagar, 2006)

Quercetin has shown anticarcinogenic activity to numerous cancer cell types, including breast, leukemia, colon, ovary, squamous cell, endometrial, gastric, and non-small-cell lung. (Painter , 1998)

Pharmacokinetics

Most animal and human trials of oral dosages of quercetin aglycone show absorption in the vicinity of 20 percent. A single intravenous dose in humans of 100 mg led to a serum quercetin concentration of 12μM (4.1 mcg/ml) (Lamson and Brignall, 2000).

Humans fed fried onions containing quercetin glucosides equivalent to 64 mg of the aglycone form reached a maximum serum concentration of 196 ng/ml 0.6 microM) 2.9 hours after ingestion. The half-life of this dose was 16.8 hours, and significant serum levels were noted up to 48 hours post ingestion (Hollman et al., 1996).

Quercetin undergoes bacterial metabolism in the intestinal tract, and is converted into phenolic acids. Absorbed quercetin is transported to the liver bound to albumin, where some may be converted via methylation, hydroxylation, or conjugation (Painter, 1998).

Dosages

Dosage An oral dose of 400-500 mg three times per day is typically used in clinical practice. , Human studies have not shown any adverse effects associated with oral administration of quercetin in a single dose of up to four grams or after one month of 500 mg twice daily (Lamson and Brignall, 2000).

Combination with Chemotherapy

Quercetin has been shown to increase the therapeutic efficacy of cisplatin both *in vivo* and *in vitro*. In mice bearing human tumor xenografts, intraperitoneal treatment with a combination of 20 mg/kg quercetin and 3 mg/ kg cisplatin led to a significantly reduced tumor growth

compared to treatment with either drug alone (Hofmann et al., 1990). Thus treatment with 20 mg/kg quercetin was not found to be an effective single agent therapy.

An *in vitro* study using human ovarian and endometrial cancer cell lines found that addition of 0.01 to 10 microM quercetin to cisplatin caused 1.5- to 30-fold potentiation of the cytotoxic effect of cisplatin. An absence of potentiation of the effect of adriamycin or etoposide due to quercetin administration was noted (Scambia et al., 1992). Quercetin (10-100 microM) has also been shown *in vitro* to protect normal renal tubular cells from cisplatin toxicity (Kuhlman et al., 1998). An *in vitro* study showed quercetin worked synergistically with busulphan against human leukemia cell lines. Quercetin/ busulphan concentrations in 1:1 and 3:1 ratios led to demonstration of much smaller cytotoxic doses of busulphan (Hoffman R 1989). Addition of 1-10 microM quercetin to adriamycin treatment led to a dose-dependent increase in cytotoxicity compared with chemotherapy treatment alone in cultured multidrug-resistant human breast cancer cell lines (Scambia et al., 1994). Quercetin has also been shown *in vitro* to increase the cytotoxic effect of cyclophosphamide, (Hofmann et al., 1988) and to decrease resistance to gemcitabine and topotecan (Sliutz et al., 1996). These last findings are consistent with the fact that many flavonoids have been shown to decrease resistance to chemotherapy in multidrug-resistant tumor cell lines.

In Vitro Studies of Quercetin:

Table 3.2 summarizes the *in vitro* experiments which have studied the malignant cell culture growth inhibition of Quercetin. Each assay showed quercetin to significantly inhibit growth. The quercetin concentration at which tumor cell growth was inhibited by 50 percent inhibitory concentration (IC50) ranged from 7 nM to just over 100 μM.

Table 3.2 In vitro studies of Quercetin (adapted from Lamson and Brignall 2000)

Malignant cell line	IC50
Bladder	Not given
Breast (MDA-MB-435)	55microM, LC50=26 microM
Breast (MDA-MB-468)	21 microM
Breast (MDA-MB-435)	31 microM
Breast (MCF-7)	4.9 microM
Breast (MCF-7)	15 microM
Colon (HT29 and Caco-2)	45-50 microM
Colon (HT29 and Caco-2)	30-40 microM
Gastric (HGC-27, NUGC-2, MKN-7, and MKN-28)	32-55 microM
Head and neck (HTB43)	Significant inhibition above 100 microM
Head and neck (HTB43 and CCL135)	Significant inhibition above 100 microM
Leukemia (14 AML lines and four ALL lines)	Average IC50=2 microM
Leukemia (CML line K562)	59 microM
Lung (non-small-cell lines)	0.45-2.28 microM
Melanoma (MNT1, M10, M14)	7nM, 20nM, 1-10 microM
Ovarian (OVCA 433)	10 microM
Note: LD50=Dose lethal to 50% of cultured cells.	

Safety/ toxicity:

A single oral dose of up to four grams of quercetin was not associated with side-effects in humans. Single intravenous bolus doses of 100 mg were apparently well tolerated as well. Intravenous bolus of 1400 mg/m^2 (approximately 2.5 grams in a 70 kg adult) once weekly for three weeks was associated with renal toxicity in two of ten patients.

Transient flushing and pain at the injection site were noted in a dose-dependent manner.

Quercetin has also known to be mutagenic. Mutagenicity does not always imply carcinogenicity and most studies have found quercetin to have no carcinogenic activity *in vivo*. There are, however, studies that do appear to show an increased risk of tumors with quercetin administration. But the meaning of the findings of such studies are not clear, and should be interpreted cautiously. At the present time, quercetin is not classified by the National Toxicology Program (NTP) report as a human carcinogen (Painter, 1998; Lamson and Brignall, 2000).

3.1.5 Analytical methods:

There are number of methods available for estimation of etoposide in various working solutions and biological fluids. Some of them are listed below.

- Quercetin Dihydrate exhibits UV absorption maxima in 95% ethanol at 257nm with mMolar absorptivity of 19.95 at 257nm and 21.88 at 376nm (Merck Index).
- Mishra et al. (2005) reported Fluorescence and UV spectroscopic studies on quercetin binding to serum albumins.
- Septhum et al. (2007) reported UV-Vis Spectroscopic study of quercetin using alum as mordant.
- Zsila et al. (2003) reported the binding of Quercetin to Human Serum Albumin by circular dichroism, electronic absorption spectroscopy and molecular modelling methods.
- Zenkevich et al. (2007) reported the identification of the products of oxidation of quercetin by air oxygen at ambient temperature b using UV spectroscopy and HPLC.
- Webb and Ebeler (2004) reported the effect of BSA on stability of flavonoids in aqueous solution using UV Spectroscopy.
- Zheng et al. (2005) reported physicochemical and structural characterization of Quercetin-β-Cyclodextrin complexes.
- Williams et al. (2006) reported synthesis and characterization of quercetin vanadyl(IV) complexes by using spectroscopic methods such as UV–visible , Fourier transform IR, electron paramagnetic resonance.

REFERENCES

Anonymous. DNA topoisomerase II inhibitors. IARC Monographs on the evaluation of carcinogenic risk to humans. **2000**; 76:175–344.

Avila MA, Velasco JA, Cansado J, Notario V. Quercetin mediates the down-regulation of mutant p53 in the human breast cancer cell line MDA-MB468. Cancer Res. **1994**; 54(9):2424-2428.

BC Cancer Agency Cancer Drug Manual. Etoposide. **2006.**

Boik J. Natural Compounds in Cancer Therapy. Oregon Medical Press, Princeton, Minnesota. **2001**; 251-267.

Bromberg KD, Burgin AB, Osheroff N. A two-drug model for etoposide action against human topoisomerase II alpha. J Biol Chem. **2003**; 278:7406–12.

Chu CD, Xu JJ, Zhang HY, Xie Z, Ye HY. Determination of Etoposide (VP-16) concentration in plasma, after subcutaneous implantation, by reversed phase high performance liquid chromatography. Sepu. **2003;** 21:56-59.

Fortune JM, Osheroff N. Topoisomerase II as a target for anticancer drugs: when enzymes stop being nice. Proc Nucleic Acid Res Mol Biol. **2000**; 64:221–53.

Gatto B, Leo E. Drugs acting on the beta isoform of human topoisomerase II. Curr Med Chem. **2003**; 3:175–85.

Hamied YK, Nayak VG, Dhumal SN, Gaitonde CD, Bhate VR. Estimation of etoposide from injectables by spectrophotometry. Indian Drugs. **1991**; 28:487-488.

Hande KR. Topoisomerase II inhibitors. Update on cancer therapeutics. **2008**; 3:13-26.

Hoffman R, Graham L, Newlands ES. Enhanced anti-proliferative action of busulphan by quercetin on the human leukaemia cell line K562. Br J Cancer. **1989**; 59:347-348.

Hofmann J, Doppler W, Jakob A. Enhancement of the antiproliferative effect of cis-diamminedichloroplatinum(II) and nitrogen mustard by inhibitors of protein kinase C. Int J Cancer. **1988**; 42:382-388.

Hofmann J, Fiebig HH, Winterhalter BR. Enhancement of the antiproliferative activity of cis-diamminedichloroplatinum(II) by quercetin. Int J Cancer. **1990;** 45:536-539.

Hollman PCH, Gaag MVD, Mengelers MJB. Absorption and disposition kinetics of the dietary antioxidant quercetin in man. Free Rad Biol Med. **1996**; 21:703-707.

Igura K, Ohta T, Kuroda Y, Kaji KA. Reveratrol and quercetin inhibit angiogenesis in vitro. Cancer Lett. **2001**; 171:11-16.

Jasti BR, Jie Du, Vasavada RC. Characterization of thermal behavior of etoposide International Journal of Pharmaceutics. **1995**; 118:161-167.

Kim J, Liu L, Guo W, Meydani M. Chemical structure of flavonols in relation to modulation of angiogenesis and immune-endothelial cell adhesion. J. Nutr. Biochem. **2006**; 17:165–176.

Kuhlman MK, Horsch E, Burkhardt G. Reduction of cisplatin toxicity in cultured renal tubular cells by the bioflavonoid quercetin. Arch Toxicol. **1998**; 72:536-540.

Lamson DW and Brignall MS. Antioxidants and Cancer III: Quercetin. Altern Med Rev. **2000**; 5(3):196-208.

Lide DR. In: Handbook of Chemistry and Physics, Boca Raton (Ed), CRC Press, FL, **1997**; pp.3-78.

Manach C, Regerat F, Texier O. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutrition Research. **1996;** 16:517-534.

Martindale, The Extra Pharmacopoeia. **1982**; 28:208.

Mishra B, Barik A, Priyadarsini I and Mohan H. Fluorescence spectroscopic studies on binding of a flavonoid antioxidant quercetin to serum albumins. J. Chem. Sci. **2005**; 117(6):641–647.

Painter FM. Alternative Medicine Review. **1998**; 3 (2):140–143.

Reddy LH, Adhikari JS, Bilikere SRD, Sharma RK and Murthy RSR. Tumoricidal effects of etoposide incorporated into solid lipid nanoparticles after intraperitoneal administration in dalton's lymphoma bearing mice. The AAPS Journal. **2006**; 8 (2), Article 29.

Scambia G, Ranelletti FO, Panici PB. Inhibitory effect of quercetin on primary ovarian and endometrial cancers and synergistic activity with cis-diamminedichloroplatinum(II). Gyn Oncol. **1992**; 45:13-19.

Scambia G, Ranelletti FO, Panici PB. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breastcancer cell line: P-glycoprotein as a possible target. Cancer Chemother Pharmacol. **1994**; 34:459-464.

Septhum C, Rattanaphani V and Rattanaphani S. UV-Vis Spectroscopic Study of Natural Dyes with Alum as a Mordant. Suranaree J. Sci. Technol. **2007**; 14(1).

Sigma Material Safety Data Sheet.

Sigma Product information sheet.

Sliutz G, Karlseder J, Tempfer C, Orel L, Holzer G, Simon MM. Drug resistance against gemcitabine and topotecan mediated by constitutive hsp70 overexpression in vitro: implication of quercetin as sensitiser in chemotherapy. Br J Cancer. **1996**; 74:172-177.

Spoerke D. In: AltMedDex® System. Rouse J.(Eds), MicroMedex Inc., Englewood, Colorado, **2003**.

The Merck Index, 11th Ed., Entry# 8044.

Watt PM, Hickson ID. Structure and function of type II DNA topoisomerases. Biochem J. **1994**; 303:681–95.

Webb MR and Ebeler SE. Comparative analysis of topoisomerase IB inhibition and DNA intercalation by flavonoids and similar compounds: structural determinates of activity. Biochem. J. **2004**; 384:527–541.

Wen-fu Tan, Li-ping Lin, Mei-hong Li, Yi-Xiang Zhang, Yun-guang Tong, Dong Xiao, Jian Ding. Quercetin, a dietary-derived flavonoid, possesses antiangiogenic potential. European Journal of Pharmacology. **2003**; 459:255-262.

Williams PA, Ferrer EG, Salinas MV, Correa MJ, Naso L, Barrio DA, Etcheverry SB, Lezama and Rojo T. Synthesis, characterization, antitumoral and osteogenic activities of quercetin vanadyl(IV) complexes. J Biol Inorg Chem. **2006**; 11:791–801.

Yance DR and Sagar SM. Targeting angiogenesis with integrative cancer therapies. Integrative Cancer Therapies. **2006**; 5(1):9-29.

Zenkevich IG, Eshchenko AY, Makarova SV, Vitenberg AG, Dobryakov YG and Utsal VA. Identification of the Products of Oxidation of Quercetin by Air Oxygen at Ambient Temperature. Molecules. **2007**; 12:654-672.

Zheng Y, Haworth IS, Zuo Z, Chow M, Chow A. Physicochemical and structural characterization of Quercetin-β-Cyclodextrin Complexes. J Pharm Sci. **2005**; 94:1079-1089.

Zsila F, Bikadi Z and Simonyi M. Probing the binding of the flavonoid, Quercetin to Human Serum Albumin by circular dichroism, electronic absorption spectroscopy and molecular modelling methods. Biochemical Pharmacology. **2003**; 65:447-456.