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In Vitro Screening of Tumoricidal Properties of International Medicinal Herbs: Part II

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Abstract

With growing use of anticancer complementary and alternative medicines (CAMs) worldwide, there is a need to assess and screen commercially available natural products for relative tumoricidal properties under standard experimental conditions. In the current study, we screened and ranked 264 traditional Chinese and Egyptian herbal medicines for tumoricidal potency against malignant neuroblastoma *in vitro*. The data obtained show that tumoricidal potencies of plants were randomly dispersed throughout similar orders, families and genera under the Division: Magnoliophyta, class: Magnoliopsida, subclasses: Asteridae, Caryophyllidae, Dilleniidae, Hamamelididae, Magnoliidae and Rosidae. The most potent plant extracts (LC_{50} < 0.08 mg/ml) were prepared from gromwell root also known as 'Hong Tiao Zi Cao' (*Lithospermum Erythrorhizon*) Family (Boraginaceae) > beth root (*Trillium Pendulum*), Family (Liliaceae) and galbanum (*Ferula Galbaniflua*), Family (Apiaceae). Gromwell root is traditionally used in the preparation of Chinese medicinal tea. In addition, galbanum was highly regarded for its sacred and medicinal value according to ancient texts and the bible. Future research will be required to isolate and identify chemical constituents within these plants which are responsible for tumoricidal effects.

Keywords

herbs; screening; cancer; bible; galbanum; beth root; *Lithospermum erythrorhizon* root

INTRODUCTION

There is an increase of individuals seeking self-administration of complementary and alternative medicine (CAM)s worldwide to aid in the fight against cancer. The term CAM generally refers to the use of potential holistic therapeutic practices that contribute to the integrated health of mind, body and spirit. While a number of studies provide helpful statistics on the types of individuals who use CAM modalities (Ferrucci *et al.*, 2009; Owens *et al.*, 2009), there continues to be a lack of established research evaluating the relative efficacy of various CAMs to treat or assist in the treatment of cancer.

With regard to chemoprevention, popular consumer choices are known to include the oral administration of antioxidant supplements, glutamine, arginine, zinc, omega-3, fatty acids,

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Conflict of Interest
The authors have declared that there is no conflict of interest.

probiotics, prebiotics, garlic and phytochemical rich spices such as turmeric, red chilli, cloves, ginger, nutmeg, fennel, fenugreek and black cumin (Blot, 1997; Rosenberg *et al.*, 2002; Conney, 2003; Kraft, 2009). Once cancer is established and diagnosed, self-administration of CAMs can occur without apprising the primary care physician (Clerici *et al.*, 2009; Richardson *et al.*, 2000; Ohno *et al.*, 2009) often including oral administration of selenium, beta-carotene (van Tonder *et al.*, 2009), herbal teas, green tea (Boon *et al.*, 2000; Yates *et al.*, 2005; Scott *et al.*, 2005; Molassiotis *et al.*, 2006), mistletoe, ginseng, cayenne, chamomile, don quai, feverfew, kava kava, milk thistle, licorice, meadowsweet, motherwort, senna leaf, shepherds purse and stinging nettle (Advance Data, CDC, 2004; Dy *et al.*, 2004; Hu *et al.*, 2005; Kumar *et al.*, 2005; Gerson-Cwilich *et al.*, 2006; Melnick, 2006; Tarhan *et al.*, 2009). Although a number of reports suggest that the prevalence of self-administered CAMs is greatest when the disease prognosis is poor (Kristoffersen *et al.*, 2009) or in instances of pediatric cancers (Genc *et al.*, 2009; Clerici *et al.*, 2009), there remains meager research on the relative potencies or efficacy of CAMs utilized in late stage cancers.

In our first report entitled '*In Vitro* Screening of Tumoricidal Properties of International Medicinal Herbs' (Mazzio and Soliman, 2009), the tumoricidal potencies of the most popular plant based CAMs were evaluated and ranked. Hundreds of international medicines, herbs and plants which are distributed and available to the public worldwide were also tested. While the data showed significant tumoricidal effects for green tea, feverfew, senna leaf, nutmeg, ginger and clove, promising herbs with the lowest LC_{50}s included: wild yam root, balm of gilead bud, chapparal, frankincense and bakuchi seed. In contrast, many popular CAMs used against cancer such as chamomile, milk thistle, motherwort, shepherd's purse and fennel seed etc., showed weak, or a lack of, tumoricidal properties where the LC_{50} exceeded 5 mg/mL *in vitro*. In the current study, we continue to rank the relative efficacy of a diverse range of Chinese and Egyptian herbal medicines for tumoricidal cytotoxic properties in malignant neuroblastoma under uniform extraction and experimental conditions *in vitro*.

MATERIALS AND METHODS

Neuro-2A cells (N-2A) cells were purchased from American Type Culture Collection (Manassas, VA). Dulbecco's modified Eagle medium (DMEM), l-glutamine, fetal bovine serum – heat inactivated (FBS), phosphate buffered saline (PBS), Hank's balanced salt solution (HBSS) and penicillin/streptomycin were purchased from Fischer Scientific, Mediatech, (Pittsburgh, PA, USA). Chinese herbal medicines were purchased from Mayway Herbs (Oakland, CA) with all other herbs being obtained from Kalyx Natural Marketplace (Camden, NY, USA), Frontier Natural Brands, (Norway, Iowa, USA), Mountain Rose Herbs (Eugene, OR, USA), Scents of the Earth (Cape May, NJ) and Monterey Bay Spice Company (Santa Cruz, CA). Chemicals and research supplies were purchased from Sigma Chemical (St Louis, MO, USA).

Extraction and sample preparation

All crude plants were weighed (0.25 g), pulverized, macerated/homogenized and extracted in 1000 μL of absolute ethanol for 7 days at 4°C (Chakraborty *et al.*, 2004) in the absence of light. A stock solution for each extract was subsequently prepared by dilution to 10 mL with HBSS + 5 mM (*N*-[2-hydroxyethylpiperazine-*N*′-[2-ethanesulfonic acid]) (HEPES), pre-adjusted to a pH of 7.4. Dilutions of each experimental extract were prepared from the stock solution in order to span a 1000-fold concentration range with the highest final plating concentration set at 5 mg/mL (w/v).

Cell culture

Neuro-2A cells (N-2A) were used to screen for tumoricidal effects, as they were originally derived from a malignant spontaneous tumor and deemed appropriate for evaluation of chemotherapy drugs (Klebe and Ruddle, 1969; Finklestein *et al.*, 1975; Mazzio *et al.*, 2003). Briefly, N-2A cells were cultured in DMEM containing phenol red supplemented with 10% FBS, 4 mM L-glutamine, 20 μm sodium pyruvate and penicillin/streptomycin (100 Units/0.1 mg, mL). The cultures were maintained at 37°C in 5% CO_2/atmosphere and sub-cultured every 2–3 days. Experimental plating media consisted of DMEM (- phenol red) supplemented with 1.8% FBS, penicillin/streptomycin (100 Units/0.1 mg/mL), 20 μm sodium pyruvate and 4 mm L-glutamine. The cells were plated in 96-well plates at a density of ~0.5×10^6 cells/mL. A three tier process was established where all extracts were evaluated at 0.5–5 mg/mL (tier 1). Those inducing cell death at any level were then re-examined at (0.1–0.5 mg/mL) (tier 2), and those inducing cell death at any level of tier 2, where further evaluated at tier 3 (.05–0.1 mg/mL). Any extract that was lethal within this range was re-tested, and further assessed at lower concentrations.

Evaluation of cellular toxicity

Cell viability was assessed by resazurin-almar blue indicator dye as described previously (Mazzio *et al.*, 2003). Experimental blanks and extract controls were run simultaneously with samples, in order to detect any interferences or reactivity with the dye or cell viability. Briefly, almar blue was dissolved in sterile PBS (0.5 mg/mL) and the cell viability was assessed by quantifying the reduction of the dye to its corresponding fluorescent intermediate – resorufin. The use of fluorescence for cell viability eliminates significant interferences introduced by experimental compounds themselves, otherwise presented during UV detection using spectrophotometric dyes. The fluorescence intensity was analysed using a microplate fluorometer – Model 7620 version 5.02 (Cambridge Technologies Inc, Watertown, Mass) with settings held at [550/580], [excitation/emission].

Evaluation of cell death

Fluorescein diacetate (FD) was used to corroborate the loss of cell viability (Mazzio *et al.*, 2003). FD is cleaved by viable esterases in living cells where a loss of fluorescence is indicative of cell death. Samples were analysed photographically using an Olympus IX-70 inverted microscope and images were captured using a MD35 Electronic Eyepiece (Zhejiang Jincheng Science and Technology Co., Ltd, China) with acquisition using C-imaging systems confocal PCI-Simple software (Compix Inc. Cranberry Township, PA, USA).

Data analysis

Statistical analysis was performed using both Origin Lab Scientific Evaluation Software (version 7.5 SR6) (Original Lab Corp., Northampton, MA, USA) and Graphpad Prism (version 3.0), (Graphpad Software Inc. San Diego, CA, USA). The lethal concentrations (LC_{50}) were established from dose-dependent data with Origin Lab 7.5 SR6 and significance of difference between the groups was assessed using a one-way analysis of variance (ANOVA), followed by a Tukey post-hoc means comparison test using Graphpad Prism Ver 3.0 software.

RESULTS

The data in Table 1 list each natural product that was examined by the common name and respective LC_{50} which was calculated from dose dependent toxicity in malignant neuroblastoma across three tiers and nine concentrations ranging from 0.005–5 mg/mL (n = 4). A taxonomical cross-reference with specific Latin names, families and plant parts are

presented in Table 2. The data are listed with the most potent tumoricidal properties first and separated into five classifications based on LC_{50} where Category 1 (Table 1A) list the strongest agents LC_{50} =[0.015–0.553 mg/mL]; Category 2 (Table 1B) moderate to strong LC_{50} =[0.554–1.504 mg/mL]; Category 3 (Table 1C), moderate LC_{50} =[1.509–3.026 mg/mL]; Category 4 (Table 1D), weak to moderate LC_{50} = [3.03–4.47 mg/mL] and Category 5 (Table 1E), weak – listing those with no tumoricidal effects and an LC_{50} > 5.0 mg/mL.

The data obtained show that less than 1% of extracts screened were capable of inducing cell death at <0.1 mg/mL. The most potent plants were 'Hong Tiao Zi Cao' (*Lithospermum erythrorhizon* root) Siebold & Zucc., common name: gromwell root > (*Trillium Pendulum*) Willd, common name: beth root and (*Ferula galbaniflua*), common name: galbanum). Figure 1 (Almar blue viability test) and Fig. 2 (FD photographic validation of viability) show that the lethal effects of *Lithospermum erythrorhizon* root in tumor cells were observed at very low concentration. In order to assess the water soluble fraction of *Lithospermum erythrorhizon* root due to its general consumer use as a tea, an herbal tea was prepared by boiling powdered root in sterile water for 5 min, then brought to room temperature. The data obtained show that ethanol extracts were identical in strength to the prepared water extract where the LC_{50} of gromwell root tea was 0.014 mg/mL and the gromwell root extract was 0.015 mg/mL (data not shown).

DISCUSSION

The current study investigates a diverse range of plants for their tumoricidal properties. While *in vitro* screenings may provide valuable information regarding elucidation of potential chemotherapy agents, it should also be noted that limitations include lack of consideration as to gastrointestinal absorption, kinetics, bioavailability, tissue distribution, route of systemic circulation, catabolism and excretion, all of which contribute to efficacy *in vivo* (Lin, 1998). With respect to direct tumoricidal properties, the data in this study show the greatest potency for the following three herbs; gromwell root (*Lithospermum erythrorhizon* Siebold and Zucc.), beth root (*Trillium pendulum* Willd.) and galbanum (*Ferula galbaniflua*).

Patterns within taxonomical classifications

The results were examined to elucidate for patterns of cytotoxic potency within similar botanical categories. Similar to the results obtained from our previous work (Mazzio and Soliman, 2009), there is an inconsistent nature by which plants exert tumoricidal effects even with similar botanical categories. For example, beth root (*Trillium pendulum*) falls under the botanical classification: Division Magnoliophyta, Class Liliopsida, Order Liliales, Subclass Lilidae and Family Liliaceae. In this study, a total of 11 plant extracts were assessed under the Liliaceae family, with two extracts ranked in the strongest category (Category 1) including Zhi Mu (Genus *Anemarrhena*) Bunge., and beth root (Genus *Trillium*) Willd., with nine extracts falling in the weakest category >5 mg/mL (Category 5). A similar trend was noted for *Ferula galbaniflua* which is classified under the Division Magnoliophyta, Class Magnoliopsida, Order Apiales, Subclass Rosidae and Family Apiaceae. Of the 17 plants examined in this category, only four were identified as strong (Category 1); *F. galbaniflua*, *F. assafoetida*, *S. divaricata*, *A sinensis* root-tail, *A. gracilistylus* root-bark, four were ranked as moderate to strong (Category 2), *P. praeruptorum* root, *N. incisium* root, *L. chuanxiong*, and four as moderate (Category 3), three as weak to moderate (Category 4) and two under the weakest category <5 mg/mL (Category 5).

The data also indicate a non-systematic pattern of cytotoxicity from extracts within the same genus including those derived from *Angelica* (LC_{50} 2.4–4.1 mg/kg), *Citrus* (LC_{50} 2.317–>5

mg/kg), *Curcuma* (LC_{50} 0.27–5 mg/kg), *Dioscorea* (LC_{50} 0.89–5 mg/kg) and *Gleditsia* (LC_{50} 0.287–5). Although there was a random nature by which tumoricidal effects were observed amongst botanical classifications, the data did indicate a trend amongst extracts from genus and species under the Division Coniferophyta, Class Pinopsida, Order Pinales, where 4/5 tested had an LC_{50} < 0.676 mg/kg, including those commonly known as arbovitae, pine and juniper.

Gromwell root

In this study, the most potent plant extract was *Lithospermum erythrorhizon* Siebold & Zucc. which is classified under the Boraginaceae family (Borage). Its extract yields a red-purple pigment analogous to synthetic dyes purported for use in commercial cosmetics (Lee *et al.*, 2008). These light sensitive pigments are also attributable to the high concentration of shikonin naphthoquinones such as deoxyshikonin, shikonin, acetylshikonin, isobutylshikonin and beta-hydroxyisovalerylshikonin which vary in color according to pH (Cho *et al.*, 1999). A large number of shikonin naphthoquinones are emerging as promising chemotherapy agents with the ability to induce apoptosis in a diverse range of cancer cells (Hou *et al.*, 2006; Cui *et al.*, 2008) also having the capability to inhibit DNA toperisomerase (Ahn *et al.*, 1995) and to protect against UV damage (Ishida and Sakaguchi, 2007). Shikonins also inhibit the proliferation and migration of endothelial cells in culture and block tumor necrosis factor (TNF)-α-induced melanoma in mice (Hisa *et al.*, 1998). It is of interest to note in this study that the tumoricidal effects of *Lithospermum erythrorhizon* superseded that of other traditional Chinese medicines commonly used for the treatment of cancer, including ‘chuan xin lian’ (andrographis), ‘ya dan zi’ (brucea fruit), ‘ban zhi lian’ (barbat skullcap), ‘shan dou gen’ (bush sophora), ‘shi shang bai’ (selaginella), ‘kuan dong hua’ (colts foot), ‘pai lan’ (eupatorium) and ‘Huang qi’ known as astralagus root (Bensky *et al.*, 2004). According to the literature, the primary use for *Lithospermum erythrorhizon* root as a traditional Chinese homeopathic medicine is for maintaining the health of the heart and liver, to facilitate the passage of stools and urine and the treatment of skin boils, eczema and burns. The advised oral daily dose of this root is 3–9 g per day, indicating its use at high concentrations as has been used historically and is generally safe (Bensky *et al.*, 2004). This study also examined the water soluble fraction of *Lithospermum erythrorhizon* root by boiling the powdered root in sterile water for 5 min, and then bringing it to room temperature. The data show that water extracts were near identical in strength yielding an LC_{50} of 0.014 mg/mL vs the *Lithospermum erythrorhizon* ethanol extract having an LC_{50} of 0.015 mg/mL.

Galbanum

In this study, galbanum (*Ferula galbaniflua*) was the third most potent extract. Galbanum is a dark brown-yellow sticky resin with a distinct pungent odor classified under the botanical family Apiaceae (carrot family). The gum is derived from cutting the stem of the plant, which upon exposure to the air forms a semi-solid substance. Galbanum has been referenced in historical literature, the bible and by ancient Egyptians as a holy anointing agent and a valuable medicine. Hippocrates described its extraordinary curative powers, and it was one of the earliest drugs known to man as a stimulant, expectorant, diuretic, antispasmodic carminative, antiseptic and antiinflammatory drug. It was commonly used to treat bronchial afflictions and arthritis. The bible in Exodus 30: 34–35 makes reference to the use of galbanum and frankincense as ingredients required in the preparation of holy incense. More recently, its medicinal use was referenced in the British Pharmacopoeia 1898, named ‘Pilula Galbani Composita’ which describes a mixture of galbanum, asafetida, myrrh and glucose. Today, the resin is used primarily as an odorant or flavoring agent associated with the fragrance of must (Bajgrowicz *et al.*, 2003).

In this study, it was found that the extract of *F. galbaniflua* was ~3.5 fold more toxic to N-2A cells than *F. assafoetida* L. However, both *Ferula* species were classified in the strongest category and pre-existing reports also corroborate the substantial antitumor properties for species within this genus. Recently it was reported that the extract of *F. vesceritensis* Coss. & DR. contains a compound called lapiferin which is responsible for cytotoxic effects on human MCF-7 breast cancer cells (Gamal-Eldeen and Hegazy, 2010). Extracts derived from the roots of *F. elaeochytris* Korovin contain 6-anthraniloyljaeschkeanadiol which exerts cytotoxic properties on K562R imatinib-resistant human chronic myeloid leukemia and a dasatinib-resistant mouse leukemia cell line (Alkhatib *et al.*, 2008). Similarly, *F. szowitsiana* DC (umbelliprenin) exerts tumoricidal effects on malignant melanoma, cell lung carcinoma and prostate carcinoma (Barthomeuf *et al.*, 2008), where *F. szowitziana* DC contains conferone, a sesquiterpene coumarin known to inhibit protein transporter P-glycoprotein indicating potential in treating multidrug resistant carcinoma (Barthomeuf *et al.*, 2006). The present study reports that *F. assafoetida* L. exerts potent tumoricidal effects. These findings have also been reported where *F. assafoetida* L is known to contain ferulic acid and farnesiferols, which at very low concentration can prevent vascular endothelial growth factor initiated processes, angiogenesis and the progression of mouse Lewis lung cancer in mice (Lee *et al.*, 2010; Ghosh *et al.*, 2009). *In vitro*, terpenes and other constituents within extracts of *F. assafoetida* L. may be responsible for cytotoxic effects which at low concentrations (<4 μg/mL) are induced against cancer cell lines such as HepG2, Hep3B and MCF-7 (Lee *et al.*, 2009).

Beth root

There seems to be no existing research investigating the bio-therapeutic potential for beth root, which only recently was reported to contain steroidal saponins hypothesized to account for therapeutic efficacy in menopausal women (Hayes *et al.*, 2009). It is also likely that the steroidal glycosides within the root may be accountable for cytotoxic effects on tumor cells (Yokosuka and Mimaki, 2008). While there is a lack of existing research on this plant root, historical literature suggests a benefit for the treatment of colds, hemorrhage, diarrhea and dysentery. Future research will be required to investigate constituents in this plant primarily responsible for the lethal effects on malignant cell lines as observed in this study.

In summary, the findings from this study suggest that relative to the hundreds of other plants tested, gromwell root, bethroot and galbanum are the most cytotoxic to tumor cells at low concentrations. These plants should be further explored for anticancer constituents, application to other types of tumor cells, and could be considered for future CAM strategies that apply to suppressing the growth of malignant tumors.

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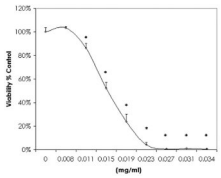

Figure 1.
The effects of gromwell root on the loss of cell viability in murine neuroblastoma cells derived from a malignant spontaneous tumor as determined with almar blue. The data are expressed as the mean ± SEM (*n* =4), and represent viability as % control.

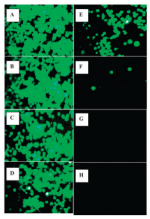

Figure 2.
The effect of gromwell root on the loss of cell viability in murine neuroblastoma cells derived from a malignant spontaneous tumor as determined by photographic acquisition of cells stained with FD. (A) Controls, (B) 0.008 mg/mL, (C) 0.011 mg/mL, (D) 0.015 mg/mL, (E) 0.019 mg/mL, (F) 0.023 mg/mL, (G) 0.027 mg/mL, (H) 0.031 mg/mL.

Table 1

The effect of natural products on cell viability in murine neuroblastoma cells originally derived from a spontaneous malignant tumor. The data represent the Common English name or Chinese name and the LC_{50} (mg/mL) calculated from 3–9 concentrations spanning a thousand-fold dilution range (n = 4)

A. Anti-Cancer Screen – Category 1: Strongest					
LC_{50} = [0.015–0.553 *mg/ml*] ●⟶					
Gromwell root	[0.015]	Burningbush	[0.375]	Cao Dou Kou	[0.531]
Beth root	[0.032]	Silk Tree [1]	[0.378]	Lilytree	[0.532]
Galbanum	[0.078]	Akebia	[0.401]	Baikal Scullcap	[0.533]
Asafetida	[0.253]	Damask Rose	[0.451]	Ji Xue Teng	[0.534]
Yuan Zhi	[0.255]	Southern Chinese Pine	[0.464]	Szechuan Pepper	[0.538]
Zhi Mu	[0.261]	California Yerba Santa	[0.485]	Official Burnet	[0.545]
Tumeric	[0.269]	Common Tansy	[0.502]	Daisy	[0.548]
White Edge Morning Glory	[0.28]	Woodland Figwort	[0.505]	Ramanas Rose	[0.549]
Locust	[0.287]	Common Hop	[0.508]	Moutain Peony	[0.549]
Chinese Rhubarb	[0.353]	Common Selfheal	[0.527]	Cinnamon Twig	[0.55]
Common Pricklyash	[0.373]	Oriental Arbovitae	[0.528]	Fang Feng	[0.553]
B. Anti-Cancer Screen – Category 2: Moderate to Strong					
LC_{50} = [0.554–1.504 *mg/ml*] →					
Dang Gui (Wei)	[0.554]	Florida fishpoison tree	[0.746]	Jing Jie	[1.084]
Korean Epimedium	[0.554]	Sassafras	[0.784]	Foetid Bugbane	[1.108]
Hou Po	[0.563]	Bi Xie	[0.89]	Oregano	[1.123]
Cow Cockle	[0.577]	Gravelroot	[0.892]	Partidgeberry	[1.126]
Clove Bud	[0.577]	Thoroughwort	[0.92]	Hu Po (Succinum Resin)	[1.164]
Himalayan Teasel Root	[0.6]	Chinese Rhubarb	[0.921]	Yin Chai Hu	[1.197]
Lesser Galangal	[0.603]	Evodia Fruit	[0.941]	Soybean	[1.214]
Wu Jia Pi	[0.61]	Qian Hu	[0.951]	Japanese Gentian	[1.237]
Hai Feng Teng	[0.61]	Sacred Lotus	[0.968]	European Dogbane	[1.263]
Fang Ji	[0.622]	Cinnamon Bark	[0.969]	Sha Ren Guang	[1.323]
Cultivated Radish	[0.627]	Qiang Hua	[0.972]	Cornmint	[1.329]
Arbovitae	[0.629]	Indian Madder	[0.982]	Chuan Hua Jiao	[1.357]
Common Mullein	[0.665]	Sappanwood	[0.983]	Japenese Persimmon	[1.377]

B. Anti-Cancer Screen – Category 2: Moderate to Strong

Huang Lian	[0.665]	Licorice Root [1]	[1.003]	Madder	[1.438]
Common Juniper	[0.676]	Yi Zhi Ren	[1.008]	Balloon Flower	[1.448]
Queens Delight	[0.682]	Red Sage	[1.027]	Licorice Root [2]	[1.478]
Cang Zhu	[0.704]	Sandalwood	[1.082]	Sang Ji Sheng	[1.49]
Cultivated Radish	[0.741]	Black Cardamom	[1.082]	Flowered Wintergreen	[1.504]

C. Anti-Cancer Screen – Category 3: Moderate

LC_{50} = [1.509–3.026 *mg/ml*] ●⟶

Largebracted Plantain	[1.509]	False Starwort	[2.249]	Hu Huang Lian	[2.571]
Hairy Agrimony	[1.519]	Mosla	[2.253]	Egyptian Senna	[2.578]
Loosestrife	[1.562]	British Yellowhead	[2.256]	Flowering Quince	[2.656]
Ze Xie	[1.601]	Balsampear	[2.262]	Tangerine (peel immature)	[2.695]
Tian San Qi	[1.8]	Sour Orange	[2.317]	Blackberry Lily	[2.723]
Chinese Corktree	[1.801]	Fu Pen Zi	[2.334]	Dang Gui (Tou)	[2.824]
Bai Zhu	[1.919]	Giant Puffball	[2.369]	Wine Grape	[2.841]
Evergreen Spice Bush	[1.943]	Japanese Pagoda Tree	[2.384]	Xiang Jia Pi	[2.901]
Florist's Daisy	[2.007]	Silk Tree [2]	[2.453]	Zhang Nao	[2.958]
Greater Burdock	[2.147]	Textile Bamboo	[2.458]	Gumweed	[2.99]
Monnier's Snowparsley	[2.159]	Du Huo	[2.471]	Canadian Wildginger	[3.026]

D. Anti-Cancer Screen – Category 4: Weak to Moderate

LC_{50} = [3.03–4.47 *mg/ml*] ●⟶

Richweed	[3.03]	Dang Gui	[3.598]	Indian Trumpet Flower	[4.087]
Marijuana	[3.071]	Chinese Violet	[3.629]	Bai Zhi	[4.122]
Poontahai	[3.071]	Licorice Root [3]	[3.761]	Oriental Arbovitae	[4.154]
Yin Chen Hao	[3.15]	Bai Jiang Cao	[3.767]	Confederate Jasmine	[4.299]
Narrowleaf Cattail	[3.166]	Mao Dong Qing	[3.773]	Bitter Lettuce	[4.315]
Cherokee Rose	[3.196]	Tangerine (seed)	[3.783]	Cape Jasmine	[4.389]
Dandelion	[3.292]	Horseradish	[3.785]	Simple Leaf Chastetree	[4.393]
Sweet Wormwood	[3.344]	Formosan Gum	[3.794]	European Centaury	[4.47]
Beefsteakplant	[3.347]	Scouringrush Horsetail	[3.929]		
Bitter Ash	[3.431]	Cocklebur	[3.935]		
Peach	[3.514]	Spike Moss	[3.987]		

D. Anti-Cancer Screen – Category 4: Weak to Moderate			
Loquat	[3.558]	Sorrell	[3.997]

E. Anti-Cancer Screen – Category 5: Weak

LC50 > [5.0 *mg/ml*]

American Plum	Da Quing Ye	Little Hogweed	Shi Chang Pu
Asiatic Dogwood	Da Zao	Locust	Siberian Ginseng
Bai Bu	Dai Zhe Shi (Haematitum)	Long Chi (Dens Draconis)	Silver Cock's Comb
Bai Dou Kou	Devils Horsewhip	Long Gu (Os Draconis)	Snake Needle Grass
Bai Fu Zi	Dill	Longan	Snow Fungus
Beefsteakplant	Duckmeat	Lou Lu	Solomon's Seal
Big Leaf Gentian	Dwarf Lilyturf	Mang Xiao (Natrii Sulfas)	Sour Orange
Bile Arisaema	E Zhu	Matrimony Vine	Spiketail
Cao Wu (Zhi)	English Walnut	Mayapple	Suan Zao Ren
Carmichael's Monkshood	European Lily of the Valley	Mexican Tea	Swallow-wort
Chaun Bei Mu	False Daisy	Ming Fan (Alumen)	Tangerine (peel)
Chi Shi Zhi (Halloysitum Rubrum	Fringed Pink	Mu Tong	Thistle
Chinese Asparagus	Fuller's Earth	No-Binu	Tian Ha
Chinese Cinquefoil	Ge Gen	Nutgrass	Tiger's Claw
Chinese Cobra Lily	Gou Teng	Paper Mulberry	Ting Li Zi
Chinese Cucumber (fruit)	Gu Jing Cao	Peach	Tuckahoe [1]
Chinese Cucumber (peel)	Herb of the Cross	Puntinpole Bamboo	Tuckahoe [2]
Chinese Cucumber (seed)	Hong Kong Lily	Qing Dai	Waxgourd
Chinese Cucumber (root)	Hua Shi (Talcum)	Qing Feng Teng	While Mulberry (fruit)
Chinese Haw	Huai Niu Xi	Qing Meng Shi (Lapis Chloriti)	While Mulberry (leaf)
Chinese Lobelia	Huang Jing	Rangoon Creeper	While Mulberry (root bark)
Chinese Motherwort	Huang Qin	Red Tangerine Peel	While Mulberry (twig)
Chinese Peony	Hyacinthbean	Ricebean (seed) [1]	Ya Dan Zi
Chinese Yam	Indian Mulberry	Ricebean (seed) [2]	Yan Hu Suo
Chuan Niu Xi	Japanese Apricot	Ricebean (root)	Yang Qi Shi (Actinolitum)
Ci Shi (Magnetitum)	Japanese Bush Cherry	Ricebean (fruit)	Yu Jin
Citron	Japanese Pagoda Tree	Sacred Lotus	Zhe Bei Mu
Cluster Mallow	Japenese Climbing Fern	San Leng	Zhu Ling
Common Barley	Ji Gu Cao	Sesame Roots	Zi Ran Tong (Pyritum)

E. Anti-Cancer Screen – Category 5: Weak			
Common Rush	Job's Tears	Sesame Seed	Zi Shi Ying (Fluoritum)
Common Wheat	Largebracted Plantain	Sha Yuan Zi	
Crowdipper	Ling Zhi (Hong)	Shan Dou Gen	

Table 2

Taxonomy of natural products listed in Table 1A–E. in alphabetical order by Common Name, *[Family]; Genus Species and Parts*

Akebia [Lardizabalaceae] Akebia trifoliata fruit
American Plum [Rosaceae] Prunus armeniaca seed
Arbovitae [Cupressaceaea] Thuja occidentalis Twigs
Asafetida [Apiaceae] Ferula Assa-Foetida
Asiatic Dogwood [Cornaceae] Cornus officinalis fruit
Bai Bu [Stemonaceae] Stemona sessilifolia root
Bai Dou Kou [Zingiberaceae] Amomum kravanh fruit
Bai Fu Zi [Araceae] Typhonium giganteum rhizome
Bai Jiang Cao [Dipsacaceae] Patrinia villosa herb
Bai Zhi [Apiaceae] Angelica dahurica root
Bai Zhu [Asteraceae] Atractylodes macrocephala rhizome
Baikal Scullcap [Lamiaceae] Scutellaria baicalensis root
Balloon Flower [Campanulaceae] Platycodon grandiflorum root
Balsampear [Cucurbitaceae] Momordica cochinchinensis seed
Beefsteakplant [Lamiaceae] Perilla frutescens fruit
Beefsteakplant [Lamiaceae] Perilla frutescens stem
Beth root [Liliaceae] Trillium pendulum root
Bi Xie [Dioscoreaceae] Dioscorea Collettii var hypoglauca rhizome
Big Leaf Gentian [Gentianaceae] Gentiana macrophylla root
Bile Arisaema [Araceae] Arisaema cum bile
Bitter Ash [Simaroubaceae] Picraena excelsa
Bitter Lettuce [Asteraceae] Lactuca virosa latex, leaves
Black Cardamom [Zingiberaceae] Amomum tsao-ko fruit
Blackberry Lily [Iridaceae] Belamcanda chinensis rhizome
British Yellowhead [Asteraceae] Inula britannica flower
Burningbush [Chenopodiaceae] Kochia scoparia fruit
California Yerba Santa [Hydrophyllaceae] Eriodictyon californicum
Canadian Wildginger [Aristolochiaceae] Asarum canadense root rhizome
Cang Zhu [Asteraceae] Atractylodes lancea rhizome
Cao Dou Kou [Zingiberaceae] Alpinia katsumadae seed
Cao Wu (Zhi) [Ranunculaceae] Aconitum kusnezoffi i root prepared
Cape Jasmine [Rubiaceae] Gardenia jasminoides fruit
Carmichael's Monkshood [Ranunculaceae] Aconitum carmichaeli root prep
Chaun Bei Mu [Ranunculaceae] Fritillaria cirrhosa bulb
Cherokee Rose [Rosaceae] Rosa Laevigata fruit
Chinese Asparagus [Liliaceae] Asparagus cochinchinensis tuber
Chinese Cinquefoil [Rosaceae] Potentilla chinensis herb
Chinese Cobra Lily [Araceae] Arisaema erubescens rhizome
Chinese Corktree [Rutaceae] Phellodendron chinense bark
Chinese Cucumber (fruit) [Cucurbitaceae] Trichosanthes kirilowii fruit

Chinese Cucumber (peel) [Cucurbitaceae] Trichosanthes kirilowii peel

Chinese Cucumber (root) [Cucurbitaceae] Trichosanthes kirilowii root

Chinese Cucumber (seed) [Cucurbitaceae] Trichosanthes kirilowii seed

Chinese Haw [Rosaceae] Crataegus pinnatifida fruit

Chinese Lobelia [Campanulaceae] Lobelia chinensis herb

Chinese Motherwort [Lamiaceae] Leonurus japonicus herb

Chinese Peony [Paeoniaceae] Paeonia lactiflora root

Chinese Rhubarb [Polygonaceae] Rheum palmatum root

Chinese Rhubarb [Polygonaceae] Rheum palmatum root & rhizome

Chinese Violet [Violaceae] Viola yezoensis herb

Chinese Yam [Dioscoreaceae] Dioscorea oppositifolia rhizome

Chuan Hua Jiao [Rutaceae] Zanthoxylum bungeanum peel

Chuan Niu Xi [Amaranthaceae] Cyathula officinalis root

Cinnamon Bark [Lauraceae] Cinnamomum cassia bark

Cinnamon Twig [Lauraceae] Cinnamomum cassia twig

Citron [Rutaceae] Citrus medica finger – fruit

Clove Bud [Myrtaceae] Eugenia caryophyllata flower bud

Cluster Mallow [Malvaceae] Malva verticillata seed

Cocklebur [Asteraceae] Xanthium sibiricum fruit

Common Barley [Poaceae] Hordeum vulgare fruit

Common Hop [Cannabaceae] Humulus lupulus

Common Juniper [Cupressaceaea] Juniperus communis

Common Mullein [Scrophulariaceae] Verbascum thapsus flower, leaf

Common Pricklyash [Rutaceae] Zanthoxylum americanum bark, berries

Common Rush [Juncaceae] Juncus effusus

Common Selfheal [Lamiaceae] Prunella vulgaris

Common Tansy [Asteraceae] Tanacetum vulgare flowering tops

Common Wheat [Poaceae] Triticum aestivum fruit

Confederate Jasmine [Apocynaceae] Trachelospermum jasminoides stem

Cornmint [Lamiaceae] Mentha haplocalyx herb

Cow Cockle [Caryophyllaceae] Vaccaria segetalis seed

Crowdipper [Araceae] Pinellia ternata rhizome

Cultivated Radish [Brassicaceae] Raphanus sativus bark

Cultivated Radish [Brassicaceae] Raphanus sativus seed

Da Quing Ye [Polygonaceae] Polygonum tinctorium leaf

Da Zao [Rhamnaceae] Ziziphus jujuba fruit-black

Daisy [Asteraceae] Chrysanthemum indicum flower

Damask Rose [Rosaceae] Rosa damascena

Dandelion [Asteraceae] Taraxacum mongolicum herb

Dang Gui [Apiaceae] Angelica sinensis root-palm sliced

Dang Gui (Tou) [Apiaceae] Angelica sinensis root-head

Dang Gui (Wei) [Apiaceae] Angelica sinensis root-tail

Devils Horsewhip [Amaranthaceae] Achyranthes aspera root

Dill [Apiaceae] Anethum graveolens seeds, leaf

Du Huo [Apiaceae] Angelica pubescens root

Duckmeat [Lemnaceae] Spirodela polyrhiza

Dwarf Lilyturf [Liliaceae] Ophiopogon japonicus tuber

E Zhu [Zingiberaceae] Curcuma wenyujin rhizome

Egyptian Senna [Fabaceae] Cassia angustifolia leaf

English Walnut [Juglandaceae] Juglans regia seed

European Centaury [Gentianaceae] Centaurium erythraea root, leaf, flower

European Dogbane [Apocynaceae] Apocynum venetum herb

European Lily of the Valley [Liliaceae] Convallaria majalis flower, leaf

Evergreen Spice Bush [Lauraceae] Lindera aggregata root

Evodia Fruit [Rutaceae] Evodia rutaecarpa fruit

False Daisy [Asteraceae] Eclipta prostrata herb

False Starwort [Caryophyllaceae] Pseudostellaria heterophylla root

Fang Feng [Apiaceae] Saposhnikovia divaricata root

Fang Ji [Menispermaceae] Stephania tetranda root

Florida fishpoison tree [Fabaceae] Piscidia erythrina root bark

Florist's Daisy [Asteraceae] Chrysanthemum morifolium flower

Flowered Wintergreen [Pyrolaceae] Pyrola calliantha herb

Flowering Quince [Rosaceae] Chaenomeles speciosa fruit

Foetid Bugbane [Ranunculaceae] Cimicifuga feotida rhizome

Formosan Gum [Hamamelidaceae] Liquidambar formosana fruit

Fringed Pink [Caryophyllaceae] Dianthus superbus herb

Fu Pen Zi [Rosaceae] Rubus chingii fruit

Galbanum [Apiaceae] Ferula Galbaniflua

Ge Gen [Fabaceae] Pueraria thomsonii root

Giant Puffball [Lycoperdaceae] Calvatia gigantea

Gou Teng [Rubiaceae] Uncaria rhynchophylla twig and thorn

Gravelroot [Asteraceae] Eupatorium purpureum root

Greater Burdock [Asteraceae] Arctium lappa fruit

Gromwell root [Boraginaceae] Lithospermum erythrorhizon root

Gu Jing Cao [Eriocaulaceae] Eriocaulon buergerianum flos

Gumweed [Asteraceae] Grindelia camporum leaf, flowering top

Hai Feng Teng [Piperaceae] Piper kadsura stem

Hairy Agrimony [Rosaceae] Agrimonia pilosa herb

Herb of the Cross [Verbenaceae] Verbena officinalis herb

Himalayan Teasel Root [Dipsacaceae] Dipsacus asperoides root

Hong Kong Lily [Liliaceae] Lilium brownii bulb

Horseradish [Brassicaceae] Armoracia rusticana root, leaf

Hou Po [Magnoliaceae] Magnolia officinalis bark

Hu Huang Lian [Plantaginaceae] Picrorhiza scrophulariaeflora rhizome

Huai Niu Xi [Amaranthaceae] Achyranthes bidentata root

Huang Jing [Liliaceae] Polygonatum sibiricum rhizome

Huang Lian [Ranunculaceae] Coptis chinensis rhizome

Huang Qin [Fabaceae] Astragalus membranaceus root

Hyacinthbean [Fabaceae] Lablab purpureus seed

Indian Madder [Rubiaceae] Rubia cordifolia root & rhizome

Indian Mulberry [Rubiaceae] Morinda officinalis root

Indian Trumpet Flower [Bignoniaceae] Oroxylum indicum seed

Japanese Apricot [Rosaceae] Prunus mume fruit

Japanese Bush Cherry [Rosaceae] Prunus japonica seed

Japanese Gentian [Gentianaceae] Gentiana scabra root

Japanese Pagoda Tree [Fabaceae] Sophora japonica flower

Japanese Pagoda Tree [Fabaceae] Sophora japonica fruit

Japenese Climbing Fern [Lygodiaceae] Lygodium japonicum spore

Japenese Persimmon [Ebenaceae] Diospyros Kaki calyx & receptacle

Ji Gu Cao [Fabaceae] Abrus cantoniensis herb

Ji Xue Teng [Fabaceae] Spatholobus suberectus vine

Jing Jie [Lamiaceae] Schizonepeta tenuifolia herb

Job's Tears [Poaceae] Coix lachryma jobi seed

Korean Epimedium [Berberidaceae] Epimedium koreanum

Largebracted Plantain [Plantaginaceae] Plantago asiatica

Largebracted Plantain [Plantaginaceae] Plantago asiatica seed

Lesser Galangal [Zingiberaceae] Alpinia offi cinarum rhizome

Licorice Root [1] [Fabaceae] Glycyrrhiza uralensis root prepared

Licorice Root [2] [Apiaceae] Ligusticum chuanxiong zhizome

Licorice Root [3] [Apiaceae] Ligusticum sinese root

Lilytree [Magnoliaceae] Magnolia denudata flower

Ling Zhi (Hong) [Gandodermataceae] Ganoderma lucidum fungus – red

Little Hogweed [Portulacaceae] Portulaca oleracea herb

Locust [Fabaceae] Gleditsia sinesis fruit

Locust [Fabaceae] Gleditsia sinesis spine

Longan [sapindaceae] Dimocarpus longan fruit

Loosestrife [Primulaceae] Lysimachia christiniae

Loquat [Rosaceae] Eriobotrya japonica leaf

Lou Lu [Asteraceae] Rhaponticum uniflorum root

Madder [Rubiaceae] Rubia tinctorum root

Mao Dong Qing [Aquifoliaceae] Ilex pubescens root

Marijuana [Cannabaceae] Cannabis sativa fruit

Matrimony Vine [Solanaceae] Lycium barbarum fruit

Mayapple [Berberidaceae] Podophyllum peltatum Root

Mexican Tea [Chenopodiac eae] Chenopodium ambrosiodes

Monnier's Snowparsley [Apiaceae] Cnidium monnieri fruit

Mosla [Lamiaceae] Mosla chinesis herb

Moutain Peony [Paeoniaceae] Paeonia suffructicose root, bark

Mu Tong [Ranunculaceae] Clematis armandii stem

Narrowleaf Cattail [Typhaceae] Typha angustifolia pollen

No-Binu [Liliaceae] Allium macrostemon bulb

Nutgrass [Cyperaceae] Cyperus rotundus rhizome

Official Burnet [Rosaceae] Sanguisorba officinalis root

Oregano [Lamiaceae] Origanum vulgare

Oriental Arbovitae [Cupressaceaea] Platycladus orientalis seed

Oriental Arbovitae [Cupressaceaea] Platycladus orientalis twig/leaf

Paper Mulberry [Moraceae] Broussonetia papyrifera fruit

Partidgeberry [Rubiaceae] Mitchella repens berries, aerial parts

Peach [Rosaceae] Prunus persica

Peach [Rosaceae] Prunus persica seed

Poontahai [Sterculiaceae] Sterculia Lychnophorum seed

Puntinpole Bamboo [Poaceae] Bambusa tuldoides shavings

Qian Hu [Apiaceae] Peucedanum praeruptorum root

Qiang Hua [Apiaceae] Notopterygium incisium root

Qing Dai [Polygonaceae] Polygonum tinctorium levis

Qing Feng Teng [Menispermaceae] Sinomenium acutum stem

Queens Delight [Euphorbiaceae] Stillingia sylvatica root

Ramanas Rose [Rosaceae] Rosa rugosa flower

Rangoon Creeper [Combretaceae] Quisqualis indica fruit

Red Sage [Lamiaceae] Salvia miltiorrhiza root

Red Tangerine Peel [Rutaceae] Citrus rubrum peel

Ricebean (fruit) [Poaceae] Orzya sativa fruit

Ricebean (root) [Poaceae] Oryza sativa root

Ricebean (seed) [1] [Phaseolus] Phaseolus calcaratus seed

Ricebean (seed) [2] [Phaseolus] Phaseolus radiatus seed

Richweed [Lamiaceae] Collinsonia canadensis root, leaf

Sacred Lotus [Nelumbonaceae] Nelumbo nucifera plumule

Sacred Lotus [Nelumbonaceae] Nelumbo nucifera seed – white

San Leng [Sparganiaceae] Sparganium stoloniferum rhizome

Sandalwood [Santalaceae] Santalum album wood

Sang Ji Sheng [Loranthaceae.] Taxillus chinensis stem & leaf

Sappanwood [Fabaceae] Caesalpinia sappan wood

Sassafras [Lauraceae] Sassafras officinale

Scouringrush Horsetail [Equisetaceae] Equisetum hyemale herb

Sesame Roots [Pedaliaceae] Sesamum indicum seeds, roots

Sesame Seed [Pedaliaceae] Sesamum indicum seed

Sha Ren Guang [Zingiberaceae] Amomum villosum fruit

Sha Yuan Zi [Fabaceae] Astragalus complanatus seed

Shan Dou Gen [Fabaceae] Sophora tonkinensis root

Shi Chang Pu [Acoraceae] Acorus tatarinowii rhizome

Siberian Ginseng [Araliaceae] Acanthopanax senticosus root

Silk Tree [1] [Fabaceae] Albizia julibrissin flower

Silk Tree [2] [Fabaceae] Albizia julibrissin bark

Silver Cock's Comb [Amaranthaceae] Celosia argentea seed

Simple Leaf Chastetree [Verbenaceae] Vitex trifolia fruit

Snake Needle Grass [Rubiaceae] Oldenlandia diffusa herb

Snow Fungus [Tremellaceae] Tremella fuciformis

Solomon's Seal [Liliaceae] Polygonatum odoratum rhizome

Sorrell [Polygonaceae] Rumex acetosella aerial parts

Sour Orange [Rutaceae] Citrus aurantium fruit – immature

Sour Orange [Rutaceae] Citrus aurauntium fruit – ripe

Southern Chinese Pine [Pinaceae] Pinus tabulaeformis modular branch

Soybean [Fabaceae] Glycine max seed-prepared

Spike Moss [Selaginellaceae] Selaginella doederleinii herb

Spiketail [Stachyuraceae] Stachyurus himalaicus

Suan Zao Ren [Rhamnaceae] Ziziphus jujuba seed – prepared

Swallow-wort [Asclepiadaceae] Cynanchum stauntonii rhizome

Sweet Wormwood [Asteraceae] Artemesia annua herb

Szechuan Pepper [Rutaceae] Zanthoxylum simulans

Tangerine (peel immature) [Rutaceae] Citrus reticulata peel-immature

Tangerine (peel) [Rutaceae] Citrus reticulata peel

Tangerine (seed) [Rutaceae] Citrus reticulata seed

Textile Bamboo [Poaceae] Bambusa textilis tabasheer

Thistle [Asteraceae] Cirsium setosum herb

Thoroughwort [Asteraceae] Eupatorium fortunei herb

Tian Ha [Orchidaceae] Gastrodia elata rhizome

Tian San Qi [Araliaceae] Panax notoginseng root

Tiger's Claw [Fabaceae] Erythrina variegata bark

Ting Li Zi [Brassicaceae] Lepidium apetalum seed

Tuckahoe [1] [Polyporaceae] Poria cocos – spirit

Tuckahoe [2] [Polyporaceae] Poria cocus

Tumeric [Zingiberaceae] Curcuma longa

Waxgourd [Cucurbitaceae] Benincasa hispida seed

White Mulberry (fruit) [Moraceae] Morus alba fruit

White Mulberry (leaf) [Moraceae] Morus alba leaf

White Mulberry (root bark) [Moraceae] Morus alba root-bark

White Mulberry (twig) [Moraceae] Morus alba twig

White Edge Morning Glory [Convolvulaceae] Pharbitis nil seed

Wine Grape [Vitaceae] Vitis vinifera seed, stem, leaf, fruit

Woodland Figwort [Scrophulariaceae] Scrophularia nodosa aerial tops

Wu Jia Pi [Araliaceae] Acanthopanax gracilistylus root-bark

Xiang Jia Pi [Asclepiadaceae] Periploca sepium root-bark

Ya Dan Zi [Simaroubaceae] Brucea javanica fruit

Yan Hu Suo [Fumariaceae] Corydalis yanhusuo rhizome

Yi Zhi Ren [Zingiberaceae] Alpinia oxyphylla fruit

Yin Chai Hu [Caryophyllaceae] Stellaria dichotoma root

Yin Chen Hao [Asteraceae] Artemisia capillaris herb

Yu Jin [Zingiberaceae] Curcuma phaeocaulis tuber

Yuan Zhi [Polygonaceae] Polygala tenufolia root

Ze Xie [Alismataceae] Alisma orientale rhizome

Zhang Nao – Camphor

Zhe Bei Mu [Liliaceae] Fritillaria thunbergii bulb

Zhi Mu [Liliaceae] Anemarrhena asphodeloides rhizome

Zhu Ling [Polyporaceae] Polyporus umbellatus