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Article

Screening for selective anticancer activity of 65 extracts of plants collected in Western Andalusia, Spain

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Abstract

In our continuous search for selective anticancer treatments, we have screened 65 extracts from 45 plants collected in several areas of Western Andalusia (Spain) for cytotoxic activity against lung cancer cells and lung normal cells. Active extracts were also tested against 11 cell lines from other tissues. An extract from the leaves of *Tetraclinis articulata* (Vahl) Mast. (Cupressaceae) showed a marked cytotoxicity (IC_{50} = 0.37 ± 0.03 µg/mL) and selectivity (selectivity index = 378.3) against the lung cancer cells; cisplatin, 5-fluorouracil and an extract from the leaves of *Taxus baccata* L. (Taxaceae) were less cytotoxic and selective.

Keywords: cancer, lung cancer, selectivity, *Tetraclinis articulata* (Vahl) Mast., Cupressaceae

1. Introduction

Despite the recent approval of new anticancer treatments, metastasis continues to be an incurable disease for most cancer patients. The limited efficacy of the existing therapies is reflected in the poor survival rates of patients with the most common metastatic cancers [1]. For example, distant metastasis occur in 57% of patients diagnosed with lung cancer, and only 5% of them survive 5 years after diagnosis [1]. Understanding why the current treatments rarely cure patients with disseminated disease is important to discover better therapies. When one treats cancer cells with specific concentrations of the available anticancer drugs and examines the cells under the microscope, one generally observes a massacre. All cancer cells die in response to most treatments. However, these drugs also kill normal cells at similar concentrations. The consequence of this limited selectivity is that patients cannot receive the drug doses needed to kill all their cancer cells; such doses would also kill their normal body cells and would be lethal. Although oncology patients generally receive the maximum tolerated doses, these doses are usually insufficient to reach the drug concentrations required to eradicate their cancer cells. The surviving cancer cells continue to proliferate and eventually lead to a fatal outcome. Finding drugs with a high selectivity towards cancer cells is crucial to develop more effective treatments for patients with metastasis [2-4].

Several plants have provided useful drugs for the treatment of a variety of cancers, including lung cancer [5-8]. For example, the diterpene paclitaxel (isolated from the bark of *Taxus brevifolia* Nutt., Taxaceae) and their semisynthetic derivative docetaxel are FDA-approved drugs for the treatment of non-small cell lung cancer. Vinorelbine, a semisynthetic analog of the vinca alkaloids (isolated from *Catharanthus roseus* G. Don., Apocynaceae), is also approved for patients with this type of cancer.

Etoposide (a semi-synthetic analogue of the natural lignane podophyllotoxin, isolated from *Podophyllum* species, Podophyllaceae) and topotecan (an analogue of the quinoline alkaloid camptotethin, isolated from *Camptotheca acuminata* Decne, Nyssaceae) are approved for patients with small cell lung cancer. Because several plants have provided useful anticancer agents, we recently used lung cancer cells and lung normal cells to evaluate the selective anticancer activity of 57 extracts from plants collected in Grazalema Natural Park (Andalusia, Spain) [9]. Using a similar experimental approach, we have evaluated the selective anticancer activity of 65 extracts from 45 new plants collected in several areas of Western Andalusia, and report the results in this communication.

2. Material and Methods

2.1. Plant material

All plants were collected by Dr. F. García between November 2012 and April 2013 in several areas of Sevilla, Cadiz and Huelva (Andalusia, Spain). Collection was non-destructive and plat specimens (5-110g) were carefully selected to avoid any damage that could affect the conservation of any species. A voucher specimen was deposited in the herbarium at the Department of Vegetal Biology and Ecology, Faculty of Biology, University of Seville. The botanical names, plant parts and voucher specimen numbers are listed in Table 1. Collection coordinates are provided in Table 1S (Supplementary Material).

2.2. Preparation of the extracts

Extracts were prepared within several hours after collecting the plants. Fresh plant material (5-110g) was extracted with 100-200 mL of ethanol / ethyl acetate / water (1:1:1) at 60°C for 1 hour by using an ultrasound water bath apparatus. After vacuum filtration, ethanol and ethyl acetate were eliminated in a rotary vacuum evaporator at 60°C. Finally, the remaining water solution was lyophilized to yield dried extracts. The extraction yield (%) for each extract (see identification number in Table 1) was: **1** (1.9%), **2** (3.0%), **3** (5.6%), **4** (4.5%), **5** (0.9%), **6** (2.9%), **7** (5.9%), **8** (4.0%), **9** (2.7%), **10** (5.5%), **11** (2.0%), **12** (2.9%), **13** (3.5%), **14** (8.5%), **15** (5.2%), **16** (7.4%), **17** (8.9%), **18** (Nd), **19** (6.7%), **20** (7.2%), **21** (2.4%), **22** (1.9%), **23** (2.4%), **24** (6.0%), **25** (14.8%), **26** (3.3%), **27** (1.0%), **28** (4.4%), **29** (6.8%), **30** (10.6%), **31** (4.9%), **32** (9.6%), **33** (4.6%), **34** (4.2%), **35** (4.0%), **36** (3.3%), **37** (4.2%), **38** (3.2%), **39** (5.9%), **40** (7.5%), **41** (5.3%), **42** (3.4%), **43** (2.7%), **44** (3.5%), **45** (2.4%), **46** (3.7%), **47** (2.6%), **48** (5.5%), **49** (0.9%), **50** (4.4%), **51** (5.5%), **52** (4.3%), **53** (5.8%), **54** (5.9%), **55** (Nd), **56** (8.4%), **57** (7.8%), **58** (5.5%), **59** (8.1%), **60** (6.5%), **61** (3.6%), **62** (10.7%), **63** (6.6%), **64** (9.0%) and **65** (9.9%). The extracts were stored in dark glass bottles and kept in a cool dark place. The first cytotoxicity experiment of the screening was carried out within the first month after preparing the extracts to avoid the possible degradation of active compounds. In the first cytotoxicity experiment, a stock solution of each extract was prepared in DMSO (100 mg/mL); a part of this solution was diluted in culture medium and immediately used to treat the cells. The stock solutions were aliquoted and frozen at -80ºC. The rest of independent cytotoxicity experiments were carried out using different aliquots to avoid freeze–thaw cycles.

2.3. Chemicals and cell lines

Cisplatin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and resazurin were obtained from Sigma. MRC-5 (human lung fibroblastic cells) and A549 (human lung adenocarcinoma cells) were purchased from European Collection of Cell Cultures. PC3 (human prostate cancer cells), MCF7 (human breast cancer cells), HeLa (human cervical carcinoma cells), NB4 (human acute promyelocytic leukemica cells), HL-60 (human acute promyelocytic leukemia cells), HepG2 (human hepatocellular carcinoma cells), SW480 (human colon adenocarcinoma cells) and U2OS (human osteosarcoma cells) were kindly provided by Dr. Helleday (Karolinska Institute, Sweden). BJ-hTERT (hTERT- immortalized foreskin fibroblast BJ cells), BJ-SV40T (SV40T-transformed BJ-hTERT cells), and BJ-RASV12 (H-RAS V12-transformed BJ-SV40T cells) were generously provided by Dr. Hahn (Dana-Farber

Cancer Institute, USA) [10]. MRC-5, A549, MCF7, HeLa, HepG2, SW480, U2OS, BJ-hTERT, BJ-SV40T and BJ-RASV12 were cultured in DMEM high glucose medium (Gibco). PC3 was grown in DMEM-F12 (Gibco). HL60 and NB4 were grown in RPMI 1640 (Gibco). All media were supplemented with 100 U/mL penicillin, and 100 µg/mL streptomycin and 10% fetal bovine serum. All cells were kept at 37°C in a humidified atmosphere containing 5% CO_2. Cell culture reagents were purchased from Thermo Fisher Scientific.

2.4. Cell viability assays

Exponentially growing cells (3,000-5,000 cells per well) were seeded in 96-well plates and were allowed to grow during 24 h. The cells were then exposed to several concentrations of the extracts or the positive controls cisplatin and 5-fluorouracil. After a treatment period of 72 h, cell viability was estimated with the MTT assay or the resazurin assay [3,11]

The MTT assay is based on the ability of viable cells to convert the MTT compound (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into an insoluble and purple formazan product. After an incubation period of the cells with the MTT and a solubilization step, the quantity of the colored product is measured with a plate reading spectrophotometer. Dead cells are metabolically inactive and cannot produce the colored product. Briefly, after the 72-h treatment period, the medium was removed, the cells were washed with PBS, and 125 µL MTT (1 mg/mL in medium) were added to each well. The plates were incubated for 4 hours to allow viable cells to transform the MTT compound into an insoluble formazan product. This insoluble compound was solubilized by adding 80 µL 20% SDS in 0.02M HCl to each well and by incubating the plates overnight at 37°C. Finally, optical densities were measured at 540 nm on a multi-well plate spectrophotometer reader.

The resazurin assay is a redox-based colorimetric or fluorometric assay based on the capacity of viable cells to reduce the blue compound resazurin into the pink, fluorescent and soluble product resorufin. The quantity of resorufin produced is proportional to the number of viable cells. After the 72-h treatment period, 100 µL resazurin in medium were added to each well (final concentration of 10 µg/mL) for 1 hour before reading the fluorescence intensity at 530/590 nm (excitation/emission) on a fluorescence microplate reader.

In both assays, cell viability was calculated as percentage in relation to untreated cells from at least three independent experiments. After estimating cell viability and calculating IC_{50} values, results were expressed as mean ± standard error of the mean (SEM), and a *t*-test (paired, two tailed) was used for statistical analysis. A *P* value >0.05 is not considered statistically significant and is not represented by any symbol. A *P* value ≤0.05 is considered statistically significant and is represented with an asterisk, two asterisks (*P* ≤0.01) or three asterisks (*P* ≤0.001). Since selectivity is the most relevant parameter to detect anticancer potential *in vitro* [2-4] selectivity indices were used to quantify this parameter. The selectivity index (S.I.) was calculated as the average of the IC_{50} value in the normal cell line (MRC-5) divided by the IC_{50} value in the cancer cell line (A549) obtained in each independent experiment [3].

3. Results and Discussion

Because patients with metastatic cancers need selective anticancer treatments, we have prepared 65 extracts from 45 plants collected in Andalusia and we have used lung cancer cells (A549) and lung normal cells (MRC-5) to screen the extracts for selective cytotoxicity with the MTT assay. Table 1 shows the botanical names, families and other pertinent information on the 45 plant species. It also shows an identification number for each extract, the IC_{50} value for both cell lines and the selectivity index. Dose-response curves for the 65 extracts are provided in Figures 1S-6S (Supporting Information). Results show that several extracts induced selective cytotoxicity towards the cancer cell line, including the

extract from the leaves of *Cascabela thevetia* (L.) Lippold (Apocynaceae) (**10**), the extract from the leaves of *Digitalis purpurea* L. (Plantaginaceae) (**18**), the extract from the bark of *Frangula alnus* Mill. (Rhamnaceae) (**27**), the extract from the whole plant *Iberis ciliata* subsp. *contracta* (Pers.) Moreno (Brassicaceae) (**37**), the extract from the seeds of *Juniperus macrocarpa* Sm. (Cupressaceae) (**39**), the extract from the bulb of *Pancratium maritimum* L. (Amaryllidaceae) (**46**), and the extract from the leaves of *Tetraclinis articulata* (Vahl) Mast. (Cupressaceae) (**58**). The anticancer drugs cisplatin and 5-fluorouracil, and an extract form the leaves of *Taxus baccata* L. (Taxaceae) (**57**) also showed selectivity against the cancer cells (Table 1). The extract from the leaves of *Tetraclinis articulata* (Vahl) Mast. (**58**) showed the most relevant activity; it was more cytotoxic against the lung cancer cells (IC_{50} = 0.37 ± 0.03 µg/mL) than against the lung normal cells (IC_{50} = 129.5 ± 64.0 µg/mL), displaying a selectivity index of 378.3. The extract from the leaves of *Taxus baccata* L. (Taxaceae) was also more cytotoxic against the lung cancer cells (IC_{50} = 0.86 ± 0.27 µg/mL) than against the lung normal cells (IC_{50} = 146.9 ± 87.8 µg/mL), displaying a selectivity index of 157.3.

Extracts **10**, **18**, **37**, **46**, **57** and **58**, and the anticancer agents cisplatin and 5-fluorouracil, were tested with the resazurin assay against eleven additional cell lines: 2 leukemia cell lines, 6 cancer cell lines derived from solid tumors of different tissues (liver, colon, bone, cervix, prostate and breast) and three genetically modified skin cell lines with increasing degree of malignancy (Table 2 and Figure 7S). The extract from the leaves of *Tetraclinis articulata* (Vahl) Mast. (**58**) displayed IC_{50} values between 0.2 and 2.1 µg/mL against seven of the eight cancer cell lines. No clear differences in cytotoxicity were found for any extract or compound in the three skin cell lines with increasing degree of malignancy (BJ-hTERT, BJ-SV40T and BJ-RASV12) [10] (Table 2).

Cardiac glycosides may be responsible for the selective cytotoxicity shown by the extracts from the leaves of *Cascabela thevetia* (L.) Lippold (**10**) and the leaves of *Digitalis purpurea* L. (**18**). We and others have previously observed that plants with cardiac glycosides, and several cardiac glycosides (e.g., digitoxin), induce potent and selective cytotoxic effects against several types of cancer cells, including lung cancer cells [11-19]. Isoquinoline alkaloids may participate in the cytotoxicity and selectivity observed for the extract from the bulb of *Pancratium maritimum* L. (**46**) [20,21]. The cytotoxicity and selectivity of our extract from the leaves of *Taxus baccata* L. (**57**) are probably mediated by different taxane-type diterpenes, including paclitaxel [22,23]. Diterpenes and monoterpenes may participate in the cytotoxicity of the extract from the leaves of *Tetraclinis articulata* (Vahl) Mast. (**58**) [24,25]. The marked selectivity against lung cancer cells shown by the extract from the leaves of *Tetraclinis articulata* (Vahl) Mast. deserves additional studies.

Conflict of Interest

The authors have no conflicts of interest.

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Table 1. Cytotoxic activity of plant extracts on lung cancer cells (A549) *versus* lung normal cells (MRC-5).

	Plant name (Family)	Part used	Voucher number (SEV)	Origin	IC_{50} (Mean ± SEM, µg/ml)		S.I.
					A549 (Cancer)	MRC-5 (Normal)	
1	*Acis autumnalis* (L.) Sweet (Amaryllidaceae)	Whole plant	284654	Sevilla	32.9 ± 9.6	59.1 ± 18.0	1.9
2	*Anagallis monelli* L. (Primulaceae)	Aerial Parts	284675	Cadiz	3.2 ± 1.4	0.6 ± 0.2	0.3
3	*Anagallis monelli* L. (Primulaceae)	Root	284675	Cadiz	7.4 ± 5.2	0.9 ± 0.5	0.3
4	*Anthyllis hamosa* Desf. (Leguminosae)	Whole plant	284685	Huelva	260.9 ± 14.7	317.7 ± 3.1	1.2
5	*Aristolochia baetica* L. (Aristolochiaceae)	Fruits	284674	Sevilla	249.1 ± 2.5	649.9 ± 345.0	2.6
6	*Aristolochia baetica* L. (Aristolochiaceae)	Leaves	284674	Sevilla	91.8 ± 70.5	66.0 ± 39.9	0.9
7	*Armeria pungens* (Link) Hoffmanns. & Link (Plumbaginaceae)	Flowering aerial parts	284687	Huelva	143.4 ± 64.3	550.7 ± 352.2	3.6
8	*Armeria velutina* Welw. ex Boiss. & Reut. (Plumbaginaceae)	Whole plant	284689	Huelva	25.7 ± 1.9	36.7 ± 0.9	1.4
9	*Campanula lusitanica* L. (Campanulaceae)	Whole plant	284667	Sevilla	223.4 ± 51.2	263.7 ± 34.2	1.3
10	*Cascabela thevetia* (L.) Lippold (Apocynaceae)	Leaves	284662	Sevilla	0.14 ± 0.02	1.6 ± 0.5	26.6
11	*Centaurea sphaerocephala* L. (Compositae)	Whole plant	284683	Huelva	134.2 ± 82.4	160.4 ± 33.8	1.7
12	*Centaurea sphaerocephala* L. (Compositae)	Flowers	284676	Cadiz	139.1 ± 40.1	108.7 ± 7.7	1.7
13	*Centaurea sphaerocephala* L. (Compositae)	Leaves	284676	Cadiz	43.1 ± 11.1	114.7 ± 14.4	3.0
14	*Cistus crispus* L. (Cistaceae)	Leaves	284660	Sevilla	52.4 ± 5.1	126.4 ± 34.3	2.5
15	*Cistus crispus* L. (Cistaceae)	Root	284660	Sevilla	24.9 ± 0.7	58.8 ± 14.8	2.1
16	*Cistus salviifolius* L. (Cistaceae)	Leaves	284653	Sevilla	56.6 ± 12.6	142.7 ± 20.4	3.0
17	*Cleome violacea* L. (Cleomaceae)	Aerial Parts	284668	Sevilla	253.9 ± 39.9	261.1 ± 3.4	1.1
18	*Digitalis purpurea* L. (Plantaginaceae)	Leaves	284691	Huelva	0.17 ± 0.15	1.06 ± 0.69	9.3
19	*Dorycnium rectum* (L.) Ser. (Leguminosae)	Flowers	284690	Sevilla	102.1 ± 40.2	182.9 ± 46.1	4.3
20	*Dorycnium rectum* (L.) Ser. (Leguminosae)	Leaves	284690	Sevilla	293.6 ± 21.2	322.1 ± 27.4	1.1
21	*Echium gaditanum* Boiss. (Boraginaceae)	Aerial Parts	284684	Huelva	196.1 ± 107.2	325.6 ± 44.0	1.1
22	*Elaeoselinum foetidum* (L.) Boiss. (Apiaceae)	Flowers	284670	Sevilla	119.0 ± 16.2	262.1 ± 8.7	2.3
23	*Elaeoselinum foetidum* (L.) Boiss. (Apiaceae)	Leaves	284670	Sevilla	246.5 ± 25.5	295.0 ± 36.4	1.2
24	*Erica arborea* L. (Ericaceae)	Bark	284657	Sevilla	32.2 ± 5.6	62.1 ± 28.9	1.8
25	*Erica arborea* L. (Ericaceae)	Leaves	284657	Sevilla	45.7 ± 7.4	158.4 ± 43.6	3.9
26	*Erophaca baetica* (L.) Boiss. (Leguminosae)	Leaves	284673	Sevilla	> 1000	> 1000	Nd
27	*Frangula alnus* Mill. (Rhamnaceae)	Bark	284680	Huelva	32.8 ± 7.4	339.3 ± 74.1	12.4
28	*Frangula alnus* Mill. (Rhamnaceae)	Leaves	284680	Huelva	28.6 ± 3.5	74.0 ± 38.5	2.4
29	*Genista hirsuta* M.Vahl (Leguminosae)	Aerial Parts	284671	Sevilla	273.6 ± 5.8	363.9 ± 27.9	1.3
30	*Halimium calycinum* (L.)	Leaves	284656	Sevilla	47.4 ± 10.0	135.4 ± 23.6	4.3

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	Plant name (Family)	Part used	Voucher number (SEV)	Origin	IC_{50} (Mean ± SEM, µg/ml)		S.I.
					A549 (Cancer)	MRC-5 (Normal)	
	K.Koch (Cistaceae)						
31	*Halimium calycinum* (L.) K.Koch (Cistaceae)	Root	284656	Sevilla	60.1 ± 17.7	101.1 ± 13.6	2.2
32	*Halimium halimifolium* (L.) Willk. (Cistaceae)	Leaves	284659	Sevilla	47.9 ± 10.9	105.9 ± 21.4	2.7
33	*Halimium halimifolium* (L.) Willk. (Cistaceae)	Root	284659	Sevilla	67.5 ± 16.0	110.2 ± 33.4	1.8
34	*Hedysarum coronarium* L. (Leguminosae)	Flowers	284677	Cadiz	230.5 ± 54.6	306.9 ± 55.1	1.4
35	*Hedysarum coronarium* L. (Leguminosae)	Fruits	284677	Cadiz	247.0 ± 3.2	294.5 ± 0.4	1.2
36	*Hedysarum coronarium* L. (Leguminosae)	Leaves	284677	Cadiz	173.9 ± 60.6	262.0 ± 32.3	2.1
37	*Iberis ciliata* subsp. *contracta* (Pers.) Moreno (Brassicaceae)	Whole plant	284688	Huelva	0.31 ± 0.06	2.31 ± 0.88	13.0
38	*Jasione montana* L. (Campanulaceae)	Whole plant	284666	Sevilla	145.0 ± 26.0	301.6 ± 69.2	2.0
39	*Juniperus macrocarpa* Sm. (Cupressaceae)	Seeds	284682	Huelva	146.1 ± 125.9	> 1000	> 20
40	*Juniperus macrocarpa* Sm. (Cupressaceae)	Aerial Parts	284682	Huelva	3.7 ± 1.9	2.8 ± 1.0	0.8
41	*Malcolmia lacera* (L.) DC. (Brassicaceae)	Whole plant	284664	Sevilla	322.3 ± 86.7	295.0 ± 5.9	1.0
42	*Malva hispanica* L. (Malvaceae)	Aerial Parts	284669	Sevilla	> 1000	703.1 ± 51.1	Nd
43	*Ononis subspicata* Lag. (Leguminosae)	Whole plant	284695	Huelva	63.9 ± 23.6	232.3 ± 29.5	4.6
44	*Ornithopus compressus* L. (Leguminosae)	Whole plant	284693	Sevilla	340.7 ± 28.4	583.8 ± 150.0	1.8
45	*Ornithopus sativus* Brot. (Leguminosae)	Whole plant	284692	Sevilla	326.6 ± 37.5	699.0 ± 262.9	2.3
46	*Pancratium maritimum* L. (Amaryllidaceae)	Bulb	284681	Huelva	3.4 ± 0.2	74.1 ± 56.1	19.7
47	*Pycnocomon rutifolium* (Vahl) Hoffmanns. & Link (Caprifoliaceae)	Leaves	284678	Cadiz	71.4 ± 40.4	70.6 ± 18.1	2.2
48	*Pycnocomon rutifolium* (Vahl) Hoffmanns. & Link (Caprifoliaceae)	Root	284678	Cadiz	262.5 ± 131,9	242.9 ± 56.7	1.1
49	*Ranunculus peltatus* Schrank (Ranunculaceae)	Whole plant	284672	Sevilla	186.7 ± 54.6	170.1 ± 58.8	0.9
50	*Rhamnus lycioides subsp. oleoides* (L.) Jahand. & Maire (Rhamnaceae)	Bark	284655	Sevilla	332.2 ± 61.9	599.4 ± 122.1	2.1
51	*Rhamnus lycioides subsp. oleoides* (L.) Jahand. & Maire (Rhamnaceae)	Leaves	284655	Sevilla	151.5 ± 16.7	421.3 ± 176.5	3.1
52	*Rhamnus lycioides subsp. oleoides* (L.) Jahand. & Maire (Rhamnaceae)	Root	284655	Sevilla	257.0 ± 76.6	442.3 ± 35.1	2.3
53	*Scrophularia frutescens* L. (Scrophulariaceae)	Whole plant	284686	Huelva	281.5 ± 9.2	297.6 ± 8.6	1.1
54	*Stauracanthus genistoides* (Brot.) G. Sampaio (Leguminosae)	Aerial Parts	284679	Huelva	278.4 ± 32.9	546.5 ± 53.5	2.0
55	*Tamarix canariensis* Willd. (Tamaricaceae)	Flowers	284650	Sevilla	212.8 ± 10.2	336.3 ± 3.6	1.6
56	*Tamarix canariensis* Willd. (Tamaricaceae)	Leaves	284650	Sevilla	92.7 ± 24.6	192.1 ± 33.0	2.2

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	Plant name (Family)	Part used	Voucher number (SEV)	Origin	IC_{50} (Mean ± SEM, µg/ml)		S.I.
					A549 (Cancer)	MRC-5 (Normal)	
57	*Taxus baccata* L. (Taxaceae)	Leaves	284621	Sevilla	0.86 ± 0.27	146.9 ± 87.8	157.3
58	*Tetraclinis articulata* (Vahl) Mast. (Cupressaceae)	Leaves	284663	Sevilla	0.37 ± 0.03	129.5 ± 64.0	378.3
59	*Teucrium fruticans* L. (Lamiaceae)	Leaves	284658	Sevilla	157.1 ± 30.5	433.0 ± 112.3	2.8
60	*Thymus mastichina* (L.) L. (Lamiaceae)	Whole plant	284694	Huelva	36.8 ± 7.7	277.7 ± 40.6	8.2
61	*Tolpis barbata* (L.) Gaertn. (Compositae)	Whole plant	284665	Sevilla	86.5 ± 28.3	43.7 ± 12.2	0.5
62	*Ulex parviflorus* Pourr. subsp. *parviflorus* (Leguminosae)	Flowers	284652	Sevilla	361.7 ± 89.6	876.1 ± 426.1	4.0
63	*Ulex parviflorus* Pourr. subsp. *parviflorus* (Leguminosae)	Leaves	284652	Sevilla	99.6 ± 34.8	270.4 ± 85.3	3.4
64	*Viburnum tinus* L. (Adoxaceae)	Fruits	284651	Sevilla	26.6 ± 6.5	65.4 ± 8.6	2.6
65	*Viburnum tinus* L. (Adoxaceae)	Leaves	284651	Sevilla	234.8 ± 58.7	568.2 ± 73.0	2.6
	Cisplatin				10.5 ± 5.5*	25.4 ± 7.4 *	4.2
	5-Fluorouracil				101.8 ± 7.7*	> 1000*	> 9.9

S.I.: selectivity index (calculated as the average of the IC_{50} value in the MRC-5 normal cell line divided by the IC_{50} value in the A549 cancer cell line obtained in each independent experiment); Nd: not determined. *Data for the anticancer drugs cisplatin and 5-fluorouracil are shown as µM.

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Table 2. Cytotoxicity of selected extracts, cisplatin and 5-fluorouracil on a panel of human cell lines.

	IC_{50} (Mean ± SEM, µg/mL)						IC_{50} (Mean ± SEM, µM)	
Cell line	**10**	**18**	**37**	**46**	**57**	**58**	**Cisplatin**	**5-FU**
HepG2	24.6 ± 4.1	13.2 ± 3.0	19.4 ± 10.4	7.0 ± 1.3	632.6 ± 106.1	18.4 ± 17.1	3.5 ± 0.8	0.3 ± 0.2
SW480	20.7 ± 5.6	18.3 ± 1.2	1.2 ± 0.3	2.1 ± 0.4	2.2 ± 0.7	2.1 ± 0.8	2.0 ± 0.5	3.4 ± 1.0
U2OS	9.2 ± 1.5	8.8 ± 0.8	1.9 ± 0.2	2.8 ± 0.7	4.0 ± 1.2	2.1 ± 0.9	4.0 ± 1.0	7.2 ± 1.0
HeLa	4.7 ± 0.1	4.9 ± 0.2	3.5 ± 0.5	5.4 ± 0.4	2.5 ± 0.4	1.3 ± 0.6	4.8 ± 0.1	66.5 ± 27.0
PC3	4.7 ± 1.5	2.6 ± 0.4	5.6 ± 2.1	5.4 ± 1.2	123.8 ± 42.7	0.50 ± 0.04	7.3 ± 4.6	1.5 ± 0.8
MCF7	10.0 ± 0.5	6.5 ± 1.0	4.0 ± 0.9	5.9 ± 0.8	37.1 ± 4.9	0.7 ± 0.1	8.0 ± 3.0	1.1 ± 0.8
NB4	2.9 ± 0.7	1.8 ± 0.5	0.5 ± 0.2	1.2 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.08 ± 0.04	2.7 ± 0.3
HL-60	3.5 ± 0.4	3.6 ± 0.3	0.8 ± 0.2	1.0 ± 0.3	3.3 ± 0.2	0.7 ± 0.3	0.2 ± 0.1	0.6 ± 0.5
BJ-hTERT	3.7 ± 0.4	2.8 ± 0.3	3.4 ± 0.5	3.4 ± 0.1	4.0 ± 0.6	0.8 ± 0.3	1.2 ± 0.4	2.6 ± 0.6
BJ-SV40T	2.9 ± 0.4	2.1 ± 0.4	2.9 ± 0.4	3.2 ± 0.4	2.8 ± 0.2	1.0 ± 0.4	0.7 ± 0.2	5.8 ± 0.7
BJ-RASV12	2.9 ± 0.4	1.8 ± 0.7	4.0 ± 1.4	2.0 ± 0.7	3.3 ± 0.2	0.6 ± 0.2	1.1 ± 0.1	1.7 ± 0.8

HepG2: human hepatocellular carcinoma; SW480: human colon adenocarcinoma; U2OS: human osteosarcoma; HeLa: human cervical carcinoma; PC3: human prostate adenocarcinoma; MCF7: human breast adenocarcinoma; NB4: human acute promyelocytic leukemia; HL-60: human acute promyelocytic leukemia; BJ-hTERT: hTERT- immortalized skin non-malignant BJ; BJ-SV40T: SV40T-transformed BJ-hTERT; BJ-RASV12: H-RAS V12-transformed BJ-SV40T. Extract numbers (**10**, **18**, **37**, **46**, **57** and **58**) can be identified from Table 1.

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Supporting Information

Table 1S. Collection coordinates of plants used in this work.

Extract	Plant name	Collection Coordinates
1	*Acis autumnalis* (L.) Sweet	37º14'22.06''N 6º11'37.85''W
2-3	*Anagallis monelli* L.	36º36´15.24"N 6º16´2.76"W
4	*Anthyllis hamosa* Desf.	37º04'25.2"N 6º41'19.68"W
5-6	*Aristolochia baetica* L.	37º14´16.68 "N 6º11´48.38"W
7	*Armeria pungens* (Link) Hoffmanns. & Link	37º04'13.73"N 6º41'16.97"W
8	*Armeria velutina* Welw. ex Boiss. & Reut.	37º02'33.33"N 6º35' 53.85"W
9	*Campanula lusitanica* L.	37º14´14.46"N 6º11´55.8"W
10	*Cascabela thevetia* (L.) Lippold	37º22'59.8"N 5º59'27.36"W
11-13	*Centaurea sphaerocephala* L.	37º05'40.18"N 6º43'37.9"W
14-15	*Cistus crispus* L.	37º14'22.06''N 6º11'37.85''W
16	*Cistus salviifolius* L.	37º14'19.74''N 6º11'40.71W
17	*Cleome violacea* L.	37º14´23.37"N 6º11´52.72"W
18	*Digitalis purpurea* L.	37º27'30.78''N 6º41'20.3''W
19-20	*Dorycnium rectum* (L.) Ser.	37º20'14.71''N 5º51'27.13''W
21	*Echium gaditanum* Boiss.	37º 04'11.69"N 6º41'17.16"W
22-23	*Elaeoselinum foetidum* (L.) Boiss.	37º14´15.72"N 6º11´50.86"W
24-25	*Erica arborea* L.	37º14'38.35''N 6º11'49.92''W
26	*Erophaca baetica* (L.) Boiss.	37º14´17.72"N 6º11´46.41"W
27-28	*Frangula alnus* Mill.	37º05'40.46"N 6º43'34.47"W
29	*Genista hirsuta* M.Vahl	37º14´16.70 "N 6º11´49.05"W
30-31	*Halimium calycinum* (L.) K.Koch	37º14'21.27N 6º11'38.12W
32-33	*Halimium halimifolium* (L.) Willk.	37º14'23.43''N 6º11' 38.77W
34-36	*Hedysarum coronarium* L.	36º36´39.92"N 6º16'46.6"W
37	*Iberis ciliata* subsp. *contracta* (Pers.) Moreno	37º04'47.25"N 6º41'13.82"W
38	*Jasione montana* L.	37º13´45.76"N 6º9´16.08"W
39-40	*Juniperus macrocarpa* Sm.	37º04'13.53"N 6º41'16.34"W
41	*Malcolmia lacera* (L.) DC.	37º13´45.76"N 6º9´16.08"W
42	*Malva hispanica* L.	37º14´16.09"N 6º11´51.89"W
43	*Ononis subspicata* Lag.	37º04'45.90"N 6º41'14.14"W
44	*Ornithopus compressus* L.	37º13´45.76"N 6º9´16.08"W
45	*Ornithopus sativus* Brot.	37º13´45.76"N 6º9´16.08"W
46	*Pancratium maritimum* L.	37º 04'11.14"N 6º41'16.29"W
47-48	*Pycnocomon rutifolium* (Vahl) Hoffmanns. & Link	36º36'12.69"N 6º15'54.45"W
49	*Ranunculus peltatus* Schrank	37º13´45.76"N 6º9´16.08"W
50-52	*Rhamnus lycioides subsp. oleoides* (L.) Jahand. & Maire	37º14'25.18"N 6º11'38.35"W
53	*Scrophularia frutescens* L.	37º04'47.25"N 6º41'13.82"W
54	*Stauracanthus genistoides* (Brot.) G. Sampaio	37º04'44.55"N 6º41'16.05"W
55-56	*Tamarix canariensis* Willd.	37º15' 45.64''N 5º59'50.9''W
57	*Taxus baccata* L.	37º22'27''N 5º59'19''W
58	*Tetraclinis articulata* (Vahl) Mast.	37º22'22.18"N 5º59'10.75"W
59	*Teucrium fruticans* L.	37º14'42.60''N 6º11'52.78''W
60	*Thymus mastichina* (L.) L.	37º02'33.33"N 6º35' 53.85"W
61	*Tolpis barbata* (L.) Gaertn.	37º13´45.76"N 6º9´16.08"W
62-63	*Ulex parviflorus* Pourr. subsp. *parviflorus*	37º13'48.06"N 6º1'31.4"W
64-65	*Viburnum tinus* L.	37º22'27''N 5º59'19''W

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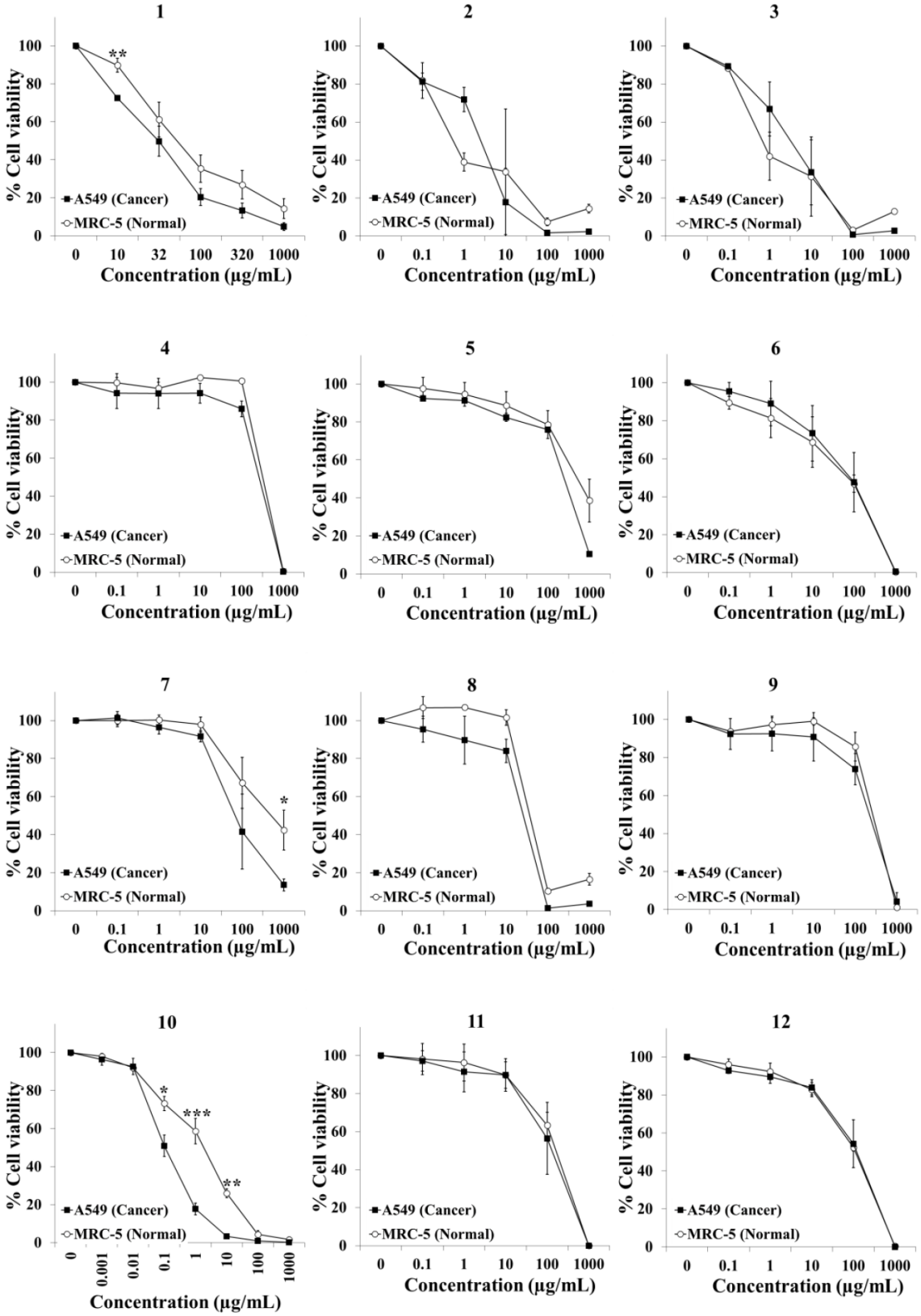

Figure 1S. Screening for selective cytotoxic activity of plant extracts **1**-**12** on A549 lung cancer cells and MRC-5 lung normal cells. The cells were exposed for 72 hours to the extracts and cell viability was determined with the MTT assay.

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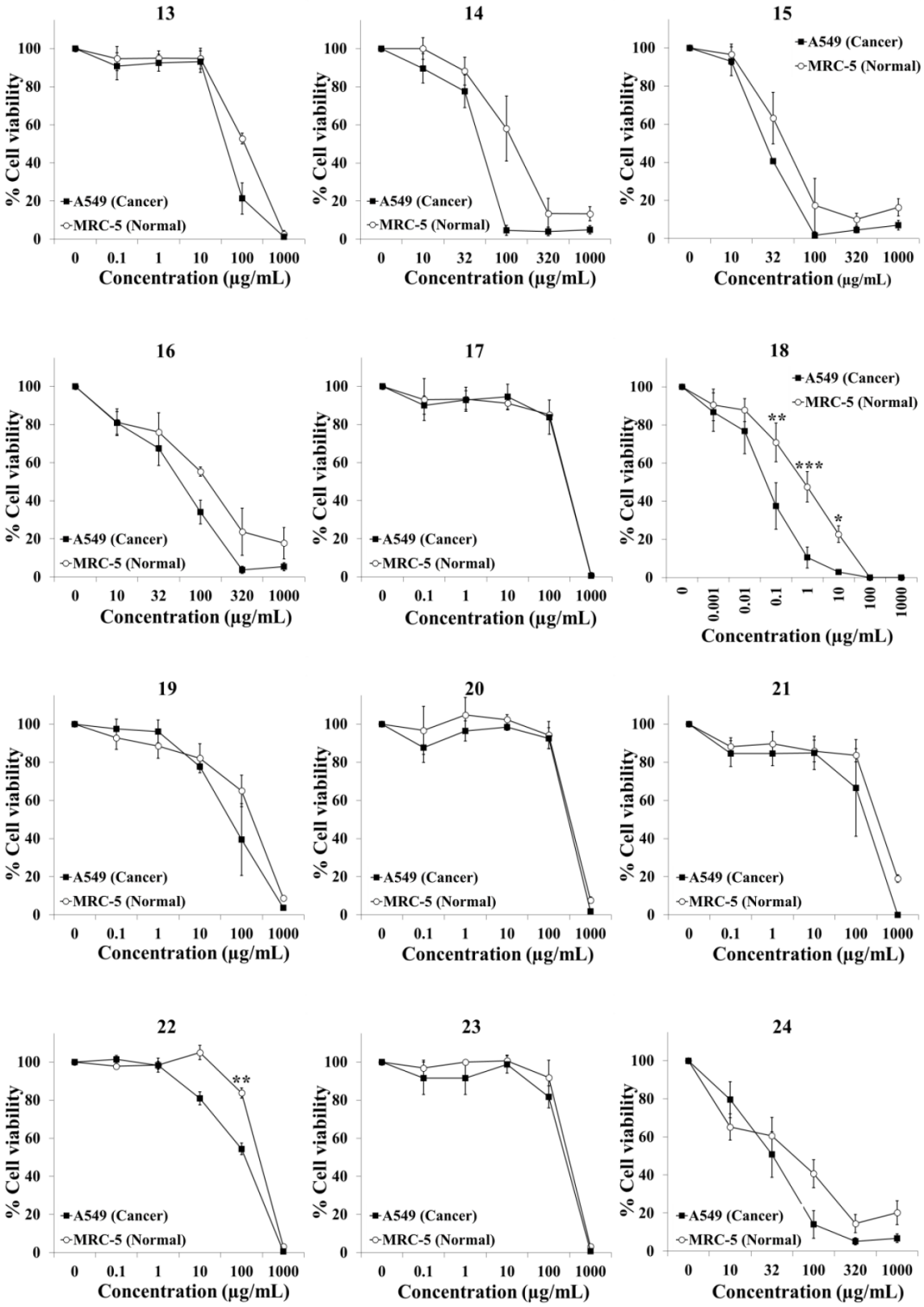

Figure 2S. Screening for selective cytotoxic activity of plant extracts **13**-**24** on A549 lung cancer cells and MRC-5 lung normal cells. The cells were exposed for 72 hours to the extracts and cell viability was determined with the MTT assay.

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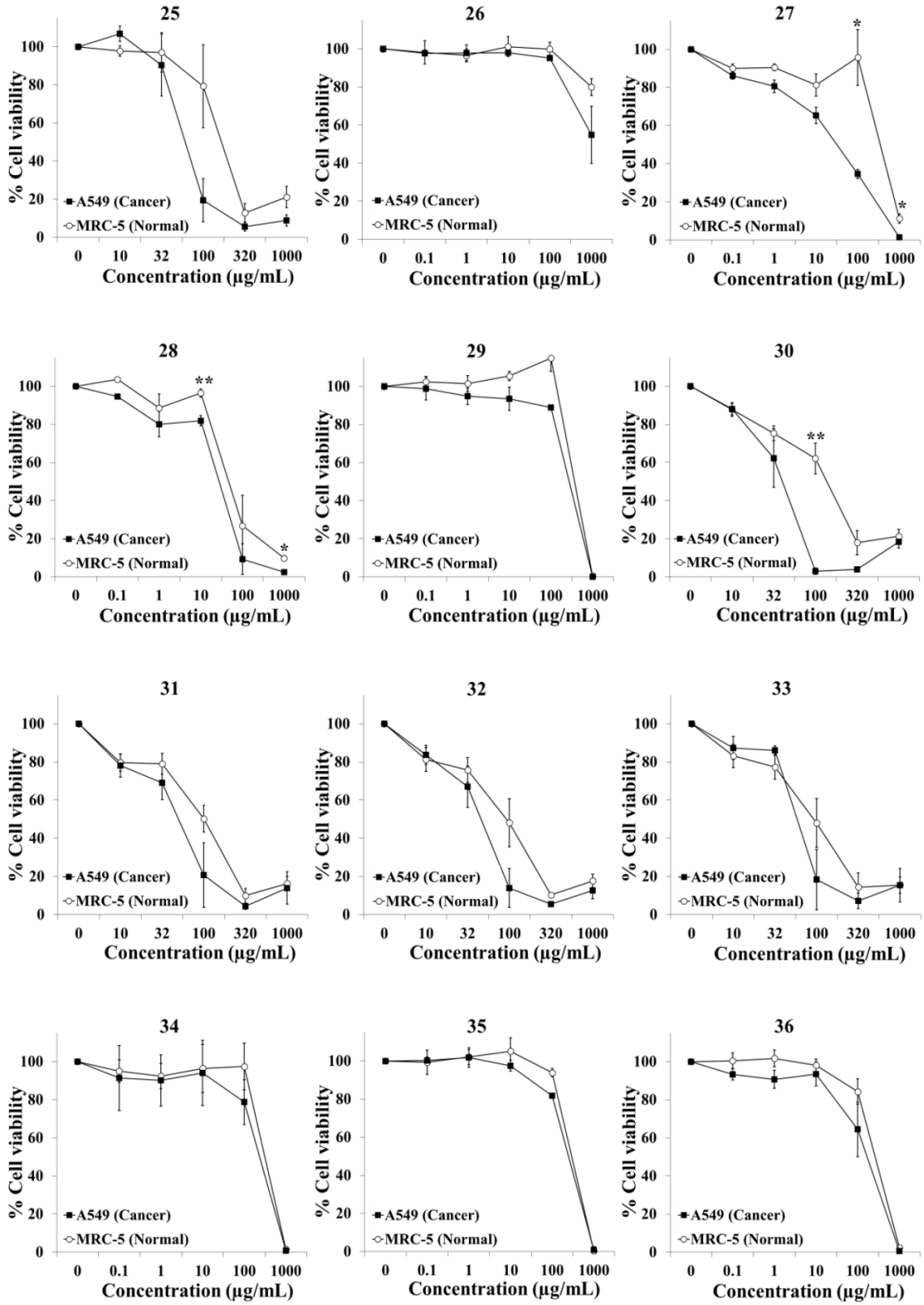

Figure 3S. Screening for selective cytotoxic activity of plant extracts **25**-**36** on A549 lung cancer cells and MRC-5 lung normal cells. The cells were exposed for 72 hours to the extracts and cell viability was determined with the MTT assay.

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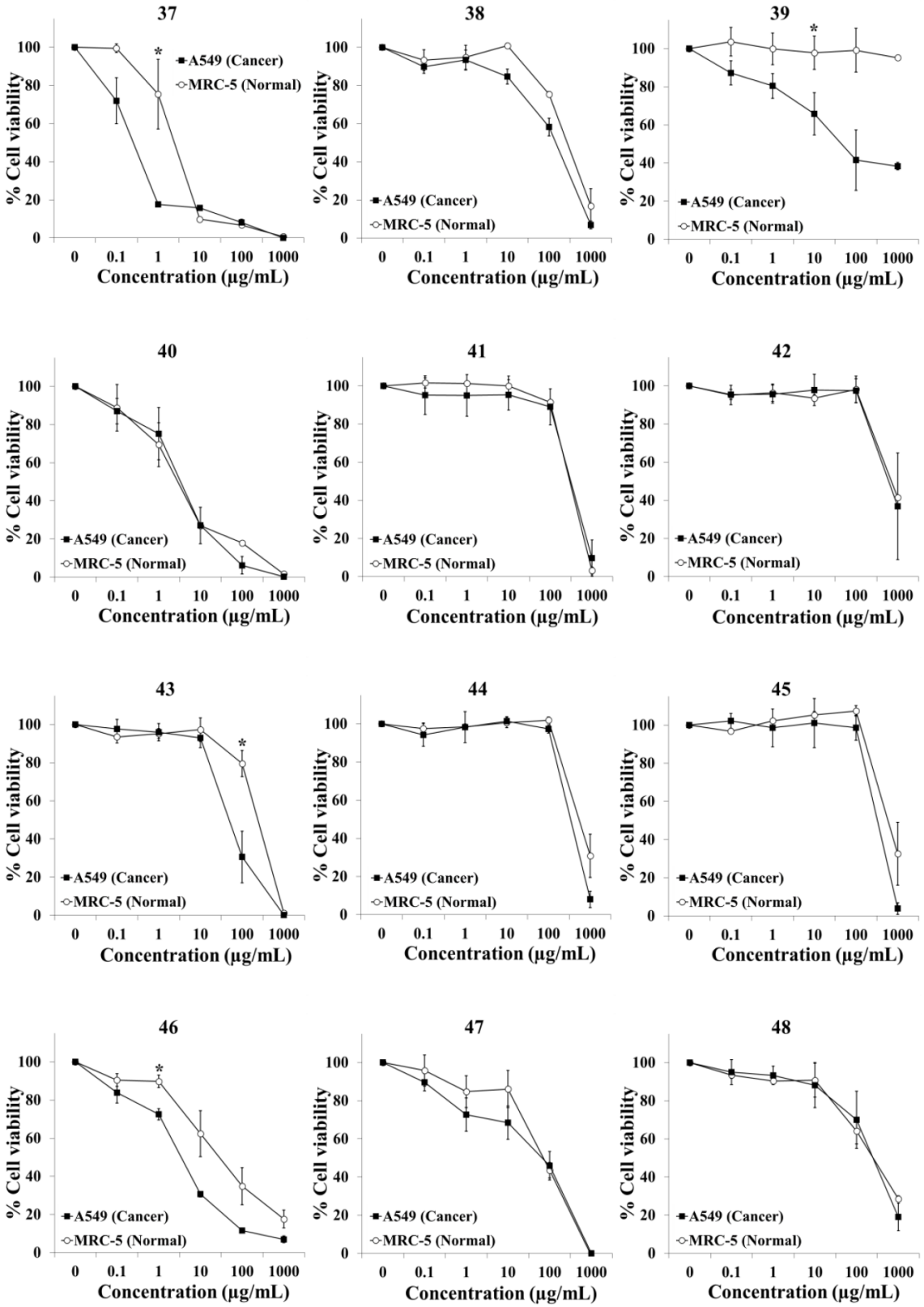

Figure 4S. Screening for selective cytotoxic activity of plant extracts **37**-**48** on A549 lung cancer cells and MRC-5 lung normal cells. The cells were exposed for 72 hours to the extracts and cell viability was determined with the MTT assay.

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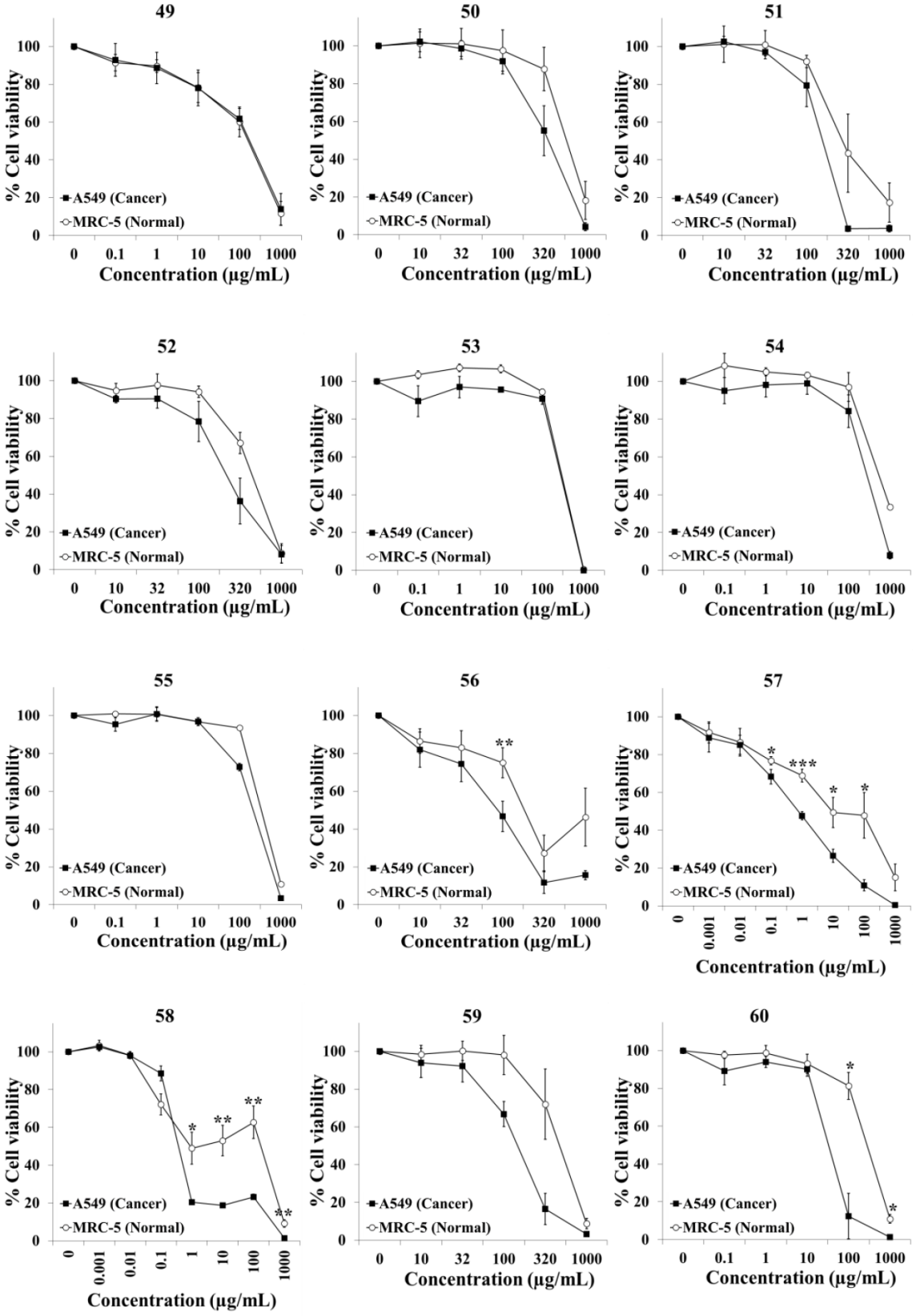

Figure 5S. Screening for selective cytotoxic activity of plant extracts **49**-**60** on A549 lung cancer cells and MRC-5 lung normal cells. The cells were exposed for 72 hours to the extracts and cell viability was determined with the MTT assay.

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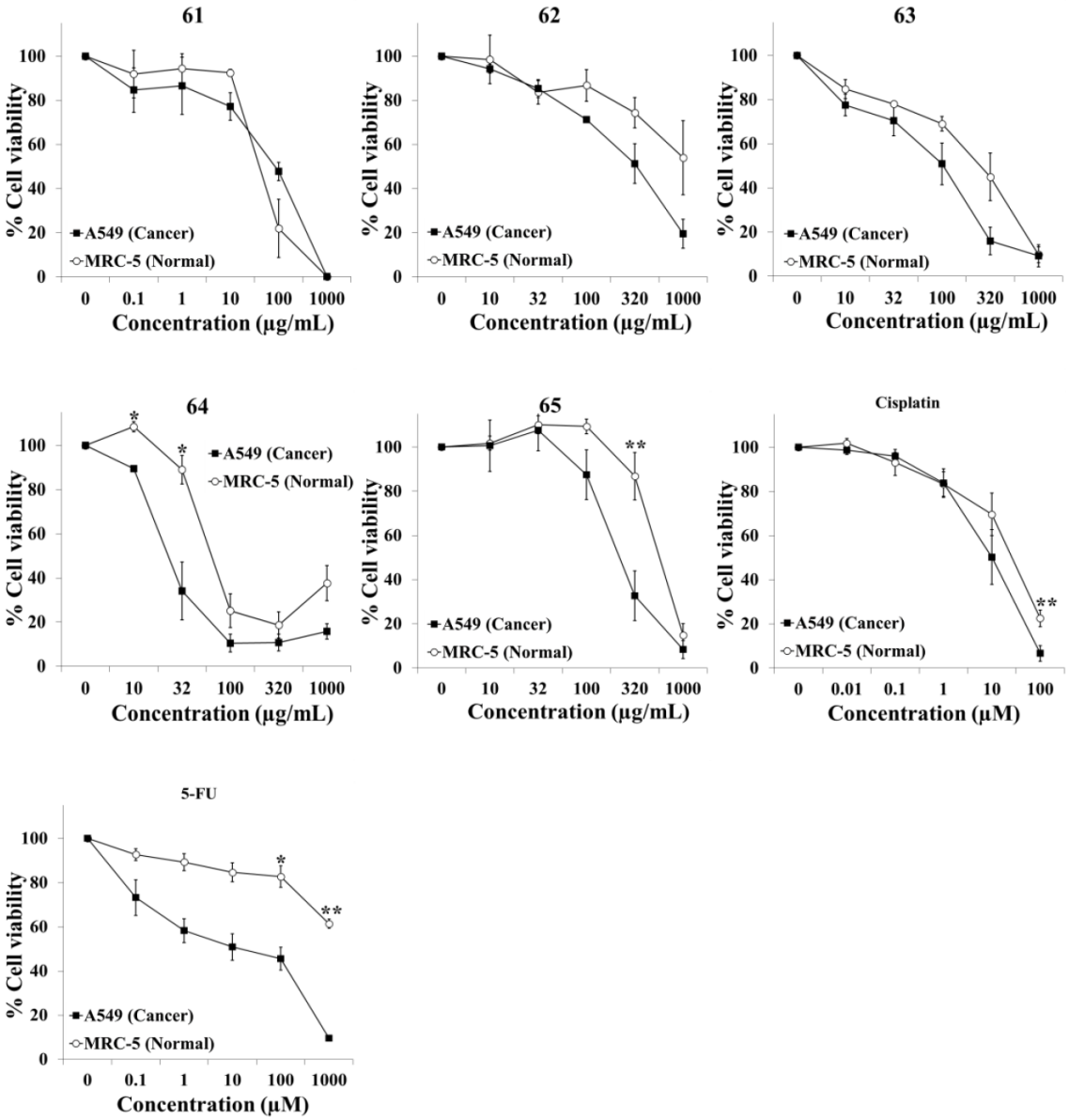

Figure 6S. Screening for selective cytotoxic activity of plant extracts **61**-**65**, cisplatin and 5-Fluorouracil (5-FU) on A549 lung cancer cells and MRC-5 lung normal cells. The cells were exposed for 72 hours to the extracts or anticancer drugs and cell viability was determined with the MTT assay.

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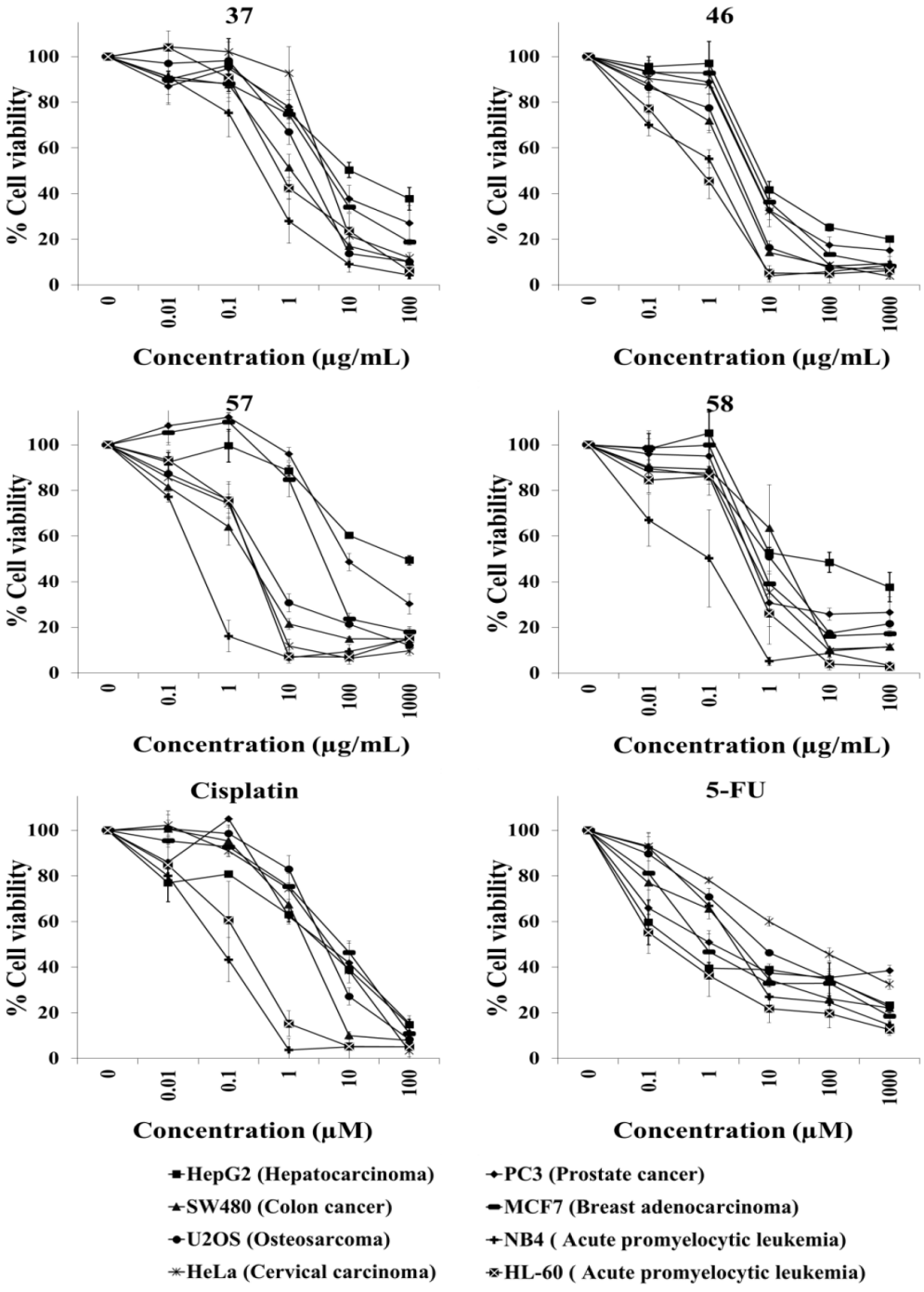

Figure 7S. Cytotoxicity of selected plant extracts (**37**, **46**, **57** and **58**), cisplatin and 5-FU against six cancer cell lines derived from solid tumors and two acute promyelocytic leukemia cell lines. Cells were treated for 72 hours and cell viability was evaluated with the resazurin assay.