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Appendix 1. Antitcancer mechanism of diosgenin.

Source	Cells/Animals/Viruses/Bacteria	Dose	Main effects/pathways	Pharmacological action	Reference
Sigma Co. (Madrid, Spain)	human squamous carcinoma cell line PE/CA-PJ15	5, 25, 50, 100 μM	boosting the G2/M phase to the detriment of the S phase	reducing cell viability, increasing apoptosis, inhibiting cell migration and modifying the cell cycle	(22)
Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India	eight to ten weeks old male golden Syrian hamsters (Mesocricetus auratus) weighing 80–120g	80 mg/kg	reducing the tumor incidence, reducing pathological changes, enhancing the process of conjugation and modulation of carcinogen metabolizing enzymes and subsequent elimination of carcinogenic metabolites, decreasing the levels of plasma and erythrocytes TBARS	antioxidant and antilipidperoxidative potential	(23)
Sigma-Aldrich (S8534)	human esophageal Eca109 cell line	50 μg/mL	inhibiting p38 protein	inhibiting cancer cell proliferation, regulating cell migration and invasion	(25)
Sigma-Aldrich (purity ≥ 98%)	human gastric cancer cells BGC-823, MGC-803	0-20 μM	inhibiting both mRNA and protein expression of MESP1	inhibiting the proliferation of gastric cancer cells	(26)
\	NCI-N87, HGC-27, MGC80-3, SGC-7901 and BGC-823	10 μM	being related to E-cadherin, integrinα5 and integrinβ6	inhibiting cancer cell invasion and survival	(27)
Sigma-Aldrich (St. Louis, MO, USA)	AGS and SGC-7901	0, 1, 2, 4, 8, 16, 32 μM	arresting G0/G1 phase, inhibiting Rho/ROCK signaling	impairing cell proliferation, increasing cell apoptosis	(29)
Qi Ling decoction (QLD)	BGC-823 and SGC-7901	0.01-10 μg/mL	inhibiting MMP-9 and affecting the PI3K/Akt signaling pathway	inhibiting the invasion, migration, and adhesion of cancer cells	(2)
Fenugreek seeds (Agriculture and Agro-Food Canada Research Center,	human colon cancer cells HT-29	0, 20, 40, 60, 80, 100 μmol/L	inhibiting bcl-2 and inducting caspase-3 protein expression	inhibiting cell growth and inducing apoptosis	(33)
	Seven-week-old male F344	1%	reducing or retarding the appearance of colonic aberrant crypt	preventing cancer	

Saskatoon, SK)	rats		foci (ACF)		
Sigma Aldrich, Saint Quentin Fallavier, France	HT-29, HCT-116 cells	0, 10, 20, 40 μM	increasing cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) expression and activity, inducing p38 MAPK pathway activation and subsequent DR5 overexpression	inducing apoptosis	(31,71)
Dioscorea floribunda	Colo-205	IC50 (27 μM)	arresting cell cycle at G1 phase and activated caspase-3	inducing apoptosis	(53)
Sigma Aldrich (St. Louis, MO, USA	SW1116, RKO	10, 20, 40, 80 μM	suppressing CRC cells through cAMP/PKA/CREB pathway, modulating p53 and Bcl-2 family proteins expression to mediate mitochondrial apoptosis pathway, reducing MMP-9, mediating glucose transporter (GLUT) like GLUT3 and GLUT4, and pyruvate carboxylase PC downregulation	increasing apoptosis, suppressing migration and invasion, decreasing aerobic glycolysis	(30)
Sigma- Aldrich (St. Louis, MO, USA). *Dioscorea floribunda*	human hepatocellular carcinoma HepG2, SMMC-7721 cells	0, 25, 50, 75, 100 μM IC50 (27 μM)	inhibiting the expression of TAZ (transcriptional co-activator with PDZ binding motif), arresting cell cycle at G1 phase and activating caspase-3, up-regulating DEAD box polypeptide 3 (DDX3), inducing G2/M phase arrest	inhibiting cell growth and proliferation, inducing apoptosis, suppressing cell migration and invasion	(17,36,53)
Fuzheng Jiedu Xiaoji formulation (FZJDXJ)	cell lines BEL7402 and MHCC97H	FZJDXJ serum	through the AKT/CyclinD1/p21/p27 pathways, retarding G1/S transition	inhibiting cell proliferation, and promoting cell apoptosis	(99)
	Nude mice (BALB/C-A, male)	a dose of 0.15 mL (64.6 mg),			
Sigma–Aldrich (St. Louis, MO)	human C3A, HepG2, HUH-7	0, 10, 20, 30, 50, 100 μM	inhibiting both constitutive and inducible activation of STAT3, suppressing activation of c-Src, JAK1 and JAK2 implicated in STAT3 activation, inducing the expression of Src homology 2 phosphatase 2 (SH-PTP2), downregulating the expression of various STAT3-regulated gene products	inhibiting proliferation and potentiating the apoptotic effects	(73)
Sigma	human HepG2 cell	0, 10, 20, 30, 40μM	resulting in activation of the caspase-3, -8, -9 and cleavage of poly-ADP-ribose polymerase (PARP) and the release of cytochrome *c*, increasing the expression of Bax, decreasing the expression of Bid and Bcl-2, and augmenting the Bax/Bcl-2 ratio, generating ROS through activation of ASK1	inducing apoptosis	(83)
Sigma-Aldrich (St Louis, MO, USA)	Pancreatic cancer Patu8988, Panc-1 cells	0, 25, 50, 75, 100 μM	reducing the expression levels of EZH2 and its target Vimentin, promoting PTEN, induced G2/M phase arrest	inducing apoptotic cell death, inhibiting cell migration and	(38)

				invasive capacities	
\	Human SCC Hep-2	40 μM	caspase-3 dependent with a fall of mitochondrial membrane potential, nuclear localization of AIF and poly (ADP-ribose) polymerase cleavage, inducing p53 activation and cell cycle arrest	inducing apoptosis, antiproliferative	(66)
fenugreek (*Trigonella foenum graecum*)	human A431, Hep2 cells, RPMI 2650, HaCat cells	2-100 μM (IC50) 40 μM	increasing the sub-G1 population, increasing Bax/Bcl-2 ratio, observing activation of caspases and cleavage of poly ADP ribose polymerase, inhibiting Akt and JNK phosphorylation	inducing apoptosis, inhibiting cell proliferation	(100)
	sarcoma 180 tumor cells in Swiss albino mice	20 mg/kg/day	reducing tumor size and tumor mass, decreasing expression of CD31	antiangiogenic, antiproliferative functions, apoptosis-promoting	
Sigma co, *foenum-graecum Linn* (fenugreek), *Dioscorea floribunda*	lung cancer cell line A549	0, 10, 20, 30,40, 50, 60 μM, IC50 (27μM)	inhibiting telomerase activity by down-regulation of hTERT gene expression, arresting cell cycle at G1 phase and activating caspase-3	inhibiting growth of cancer cell, inducing apoptosis	(45,53,82)
Sigma-Aldrich (St. Louis, MO, USA), *Dioscorea floribunda*	human prostate cancer PC-3 cells	0, 25, 50, 75, 100 μM IC50 (27μM)	inhibiting the expression of neural precursor cell expressing developmentally down-regulated protein 4 (NEDD4), arresting cell cycle at G1 phase and activatingcaspase-3	inhibiting cell growth, inducing apoptosis and cell cycle arrest	(48,53)
		0, 5, 10, 20, 30 μM	reducing MMPs expression, inhibiting ERK, JNK and PI3K/Akt signaling pathways as well as NF-kB activity	inhibiting migration and invasion	(50)
Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China) purity >98%	DU145	15, 3, 0.6 μg/mL	inhibiting the PI3K/Akt/mTOR signaling pathway	activating apoptosis and autophagy	(51)
fenugreek seed, *Dioscorea floribunda*	human breast cancer cells MCF7, MDA-MB-231 cells	0, 25, 50, 75, 100 μM, IC50 (27μM)	upregulating the expression of p57 and FOXO1, inhibiting the expression of S-phase kinase-associated protein 2(Skp2), arresting cell cycle at G1 phase and activating caspase-3	inhibiting cell viability and stimulating apoptosis, reducing cell invasion	(53,55)
Sigma-Aldrich (St. Louis, MO, USA).	MCF-7 and Hs578T	0, 5, 10, 20, 40 μM	altering phosphorylated cyclin checkpoint1 (p-Chk1Ser345) and cyclin B expression, resulting in G2/M phase blockade, down-regulating the anti-apoptotic protein, Bcl-2, releasing cytochrome *c* and activating the caspase signaling cascade	mitochondria-mediated apoptosis in cancer cells, affecting cell death	(56)

Dioscorea composita	cervical cancer cell lines HeLa and CaSki	0-80 μM	presenting compact nuclei and apoptotic bodies, positive for active caspase-3	increasing antiproliferative activity, inducing apoptosis	(58)
Nanjing Spring & Autumn Biotech Co. Ltd (purity >98%)	chronic myeloid leukemia (CML) cell lines K562, BaF 3-WT	0, 5, 10, 15, 20, 30 μM	generating ROS, inhibiting mTOR pathway	inducing autophagy and apoptosis	(65)
1.*Solanum lyratum* Extracts (Dongpu, Sinyi Township, Nantou County, Taiwan) 2.Sigma-Aldrich Corp. (St.Louis, MO,USA)	murine myelomonocytic leukemia cell line (WEHI-3)	1.0, 100, 200, 400μg/mL 2.0, 25, 50, 100 μM	increasing the protein level of p53 and decreasing the protein levels of CDK4, CDK6, and cyclin D, increasing the death receptor pathway-associated Fas/CD95, FasL, FADD, and cleavage-caspase-8 protein levels, increasing cytochrome *c*, Apaf-1, Bax, Bad, and cleavage-caspase-9, decreasing the levels of Bcl-2 and Bcl-xl	inhibiting cell proliferation, promoting G0/G1 phase arrest, inducing cell death	(64)
Solanum lyratum Extracts	BALB/c mice (4–6 weeks of age)	5, 15 mg/kg	reducing tumor volume	inhibiting tumor growth	
Dioscorea floribunda	Leukaemia Jurkat	IC50 (27 μM)	arresting cell cycle at G1 phase and activated caspase-3	inducing apoptosis	(53)
	Ehrlich ascites carcinoma in mice	50, 75 mg/kg	reducing 54.4% and 65.4% Ehrlich ascites carcinoma in mice	inhibiting tumor growth	
Nanjing Zelang Medical Technology Co., Ltd. (purity >90%)	Human osteosarcoma MG63 and U2OS cells	MG63 (80 μM) and U2OS (40 μM)	inhibiting the phosphorylated p38 (pP38) protein and the p38MAPK signaling pathway	reducing metastasis	(68)
Nanjing Spring & Autumn Biotech Co., Ltd (purity > 98%)	C57BL/6-derived melanoma cell line B16F10	0, 10, 15, 20, 25, 30 μM	inducing cell viability reduction and obvious morphological alteration in cancer cells, eliciting augmented T-cell responses	inhibiting the growth	(67)
	melanoma cells M4Beu	40 μM	caspase-3 dependent with a fall of mitochondrial membrane potential, nuclear localization of AIF and poly (ADP-ribose) polymerase cleavage, inducing p53 activation and cell cycle arrest	inducing apoptosis, antiproliferative	(66)
Nanjing Spring & Autumn Biotech Co., Ltd (purity > 98%)	C57BL/6 mice melanoma-bearing	20 mg/kg, 0.2 mL	CD4+/CD8+ T-cell infiltration and IFN-γ expression in tumor tissues, improving the compositions of intestinal microbiota	triggering tumor necrosis, apoptosis, and activation of antitumor immunity	(67)

Sigma Aldrich (St. Louis, MO, USA)	human CCA cell lines HuCCT1, QBC939, SK-ChA-1, HuH28, RBE, and Mz-ChA-1	0, 10, 20, 40, 80 μM	decreasing the expression of mitosis-promoting factor cyclinB1 along with the elevating level of cell cycle inhibitor p21, resulting in arresting CCA cell cycles at G2/M phase, cleaved-caspase-3, cleaved-PARP1 and the Bax/Bcl-2 ratio, increasing expressions of cytosol cytochrome *c*	inducing apoptosis	(39)
	Nude mice (BALB/c)	50 mg/kg	down-regulating PCNA and Ki67 protein expression	inhibiting tumor growth	

Appendix 2. Clinical applications of diosgenin in different cancers.

Source	Cases	Age (year)	Formula	Dose	Results	References
Fuzheng Jiedu Xiaoji formulation (FZJDXJ)	291 HCC patients	54.45	Codonopsis pilosula (15g), Poria cocos (15g), Astragalus membranaceus (15g), Atractylodes macrocephala (15g), Angelica sinensis (15g), Adenophora stricta (15g), Radix ophiopogonis (15g), Rehmannia glutinosa (15g), Paris polyphylla (15g), Curcuma phaeocaulis (15g), Pinellia ternata (9g)	300 ml of the herb concoction, one dose granule of FZJDXJ was added to 150 mL herbs	prolonging OS and PFS, reducing the mortality rate of HCC patients	(99)
Chinese herbal extract, specifications: 200mg/ slice	cervical cancer (n=1), esophageal cancer (n=1), lung cancer (n=10), bladder cancer (n=1), breast cancer (n=9), lymphoma (n=3), liver cancer (n=2), stomach cancer (n=2), kidney cancer (n=1), cholangiocarcinoma (n=1), bowel cancer (n=3)	38~60	containing 200 mg of diosgenin	400, 800, 1400, 2000, 2600 mg	There were no adverse events in the single administration group of diosgenin, while in the continuous dose group of 2000 mg, there were 1/6 subjects with abnormal liver function and could recover by themselves.	(104)
Chinese herbal extract, specifications: 200mg/ tablet	Breast cancer (n=3), lung cancer (n=3), rectal cancer (n=2), cervical cancer (n=2), malignant transformation (n=2), renal cell carcinoma (n=2), gastric	36~68	containing 200 mg of diosgenin	600, 1200, 2400 mg	Diosgenin has poor oral absorption rate (about 5 h to peak) and long $t_{1/2}$ (average 30 h), Prototype plasma	(103)

	cancer (n=2), prostate cancer (n=2), lymphoma (n=2), nodular thyroid tumor (n=1), thyroid cancer (n=1), uterine cancer (n=1) brain tumor (n=1).				concentration is not high. Bioavailability decreased after taking diosgenin of the high dose, the single oral of 1200 mg to 2400mg showed the process of nonlinear dynamics. The single oral of 600mg to 2400mg has good security in clinical.	
Shenlingbaizhu powder	lung cancer (n=29), bowel cancer (n=12), gastric cancer (n=7), cervical cancer (n=3), prostate cancer (n=2), pancreatic cancer (n=2), ovarian cancer (n=1), endometrial cancer (n=1), kidney cancer (n=1), liver cancer (n=1), glioma (n=1), fibrosarcoma (n=1), gallbladder cancer (n=1), cholangiocarcinoma (n=1), multiple myeloma (n=1)	35~75	Raw ginseng (20 g), Poria cocos (20 g), fried Baizhu (20 g), fried White lentil (15 g), Coicis seed (10 g), Chinese yam (20 g), fructus amomi (10 g), platycodon grandiflorum (10 g), lotus seed (10 g), roasted licorice (20 g), red date (5 g)	1 dose a day, divided into 3 times of warm administration, treatment for 4 weeks	Shenlingbaizhu powder combined with nutritional support can improve life quality and KPS, decrease TNF-α, TWEAK, Fn14 expression of the tumor cachexia patient. Shenlingbaizhu powder control cancer cachexia maybe due to its regulation of these cytokines.	(101)