Molecular mechanisms and associated cell signalling pathways underlying the anticancer properties of phytochemical compounds from *Aloe* species (Review)

HANNA SVITINA[1,2], JOSIAS H. HAMMAN[1] and CHRISNA GOUWS[1]

[1]Centre of Excellence for Pharmaceutical Sciences (Pharmacen™), North-West University, Potchefstroom, North West 2520, South Africa; [2]Department of Functional Genomics, Institute of Molecular Biology and Genetics of NASU, Kyiv 03143, Ukraine

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Abstract. Naturally occurring components from various species of *Aloe* have been used as traditional folk medicine since the ancient times. Over the last few decades, the therapeutic effects of extracts and phytochemical compounds obtained from *Aloe vera* have been proven in preclinical and clinical studies. Recently, compounds from other *Aloe* species apart from *Aloe vera* have been investigated for the treatment of different diseases, with a particular focus on cancer. In the present review, the effects of phytochemical compounds obtained from different *Aloe* species are discussed, with a specific focus on the effects on cell signalling in cancer and normal cells, and their selectivity and efficacy. This information will be useful for the application of *Aloe*-derived compounds as therapeutic agents, either alone or in combination with other standard drugs for cancer treatment.

Contents

Correspondence to: Dr Hanna Svitina, Centre of Excellence for Pharmaceutical Sciences (Pharmacen™), North-West University, Private Bag X6001, 11 Hoffman Street, Potchefstroom, North West 2520, South Africa
E-mail: anja.onishchenko@gmail.com

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1. Introduction

The genus *Aloe* belongs to the family of Xanthorrhoeaceae and includes ~400 xerophyte plant species grown all over the world. Due to their ability to survive in hot and dry conditions, *Aloe* plants tend to store water and important chemical constituents in their swollen and succulent leaves, which makes them a unique source of phytochemicals (1).

Combinations of active molecules extracted from *Aloe* species have been indicated to confer a variety of biological effects with different mechanisms of action (2). The most extensively studied *Aloe* species is *Aloe vera* (*A. vera*), the official name of which is *A. barbadensis*, which has been used as a form of medicine since the ancient times. In the period from 2014 to 2019, 35 clinical trials have been initiated to study the clinical efficacy of *A. vera* plant extracts (2). The *A. vera* plant has an estimated annual commercial market value of $US 13 billion (3,4). *A. vera* has a large number of reported phytochemical constituents that are able to improve skin disorders (5), diabetes (6,7), fibrosis (8), periodontitis (9), bone regeneration (10) and irritable bowel syndrome (11) through different mechanisms of actions. As pointed out by The International Aloe Science Council (https://www.iasc.org), extracts from other *Aloe* species, including *A. arborescens, A. arborescence var. nataliensis, A. mutabilis, A. ferox, A. candelabrum, A. africana* and *A. spicata*, also possess medicinal properties (12-17).

A previous randomised clinical trial performed by Lissoni *et al* (12) investigated 240 patients with different types metastatic solid tumour who received chemotherapy alone or chemotherapy in combination with orally administered *A. arborescens*. This study indicated that *A. arborescens* combined with chemotherapy was more effective in terms of the degree of oncostatic effect, tumour regression rate and survival time, in addition to reductions in adverse events.

Furthermore, the patient group who received *Aloe* exhibited prolonged survival times and had an improved quality of life, particularly in terms of asthenia relief and fatigue (12).

The effects of phytochemicals on cell signalling are currently a topic of intense research (18-21). Of note, medicinal plants have been the source of numerous anticancer drugs that are currently used in chemotherapy, including vincristine, irinotecan, etoposide and paclitaxel (22). Therefore, interest in the potential anticancer activity of herbal compounds is gaining attention (23). Approximately 20 chemical constituents have been reported as the major biologically active components within the extracts of *Aloe* species (24-27). These phytochemicals include anthraquinones (aloe-emodin, emodin and chrysophanol), anthranols (aloin A, aloin B, aloinoside A, aloinoside B and aloesaponol), chromones (aloesin and aloesone), coumarins (esculetin and umbelliferone), pyrans and pyrones (aloenin), flavonoids (isovitexin), naphthalene derivatives (plicataloside) and polysaccharides (acemannan) (24-27). The potential pharmacological benefits of the bioactive components of plants belonging to the *Aloe* genus have been reviewed in a recent publication (28). Aloe-derived phytochemicals have been successfully applied for treating a broad range of conditions. Indeed, *A. vera* extract has been previously demonstrated to confer renoprotective action (29), radioprotective potential (30) and virustatic effects (31). In addition, aloe- emodin has been documented to ameliorate renal interstitial fibrosis (32), whilst aloin demonstrated selective effects against *Leishmania aethiopica* and *Leishmania donovani* (33) and isovitexin was indicated to be an anti-diabetic (34,35) and neuroprotective (36) compound. Acemannan may also be used as a matrix for bone regeneration (37-39).

The present review discusses the different signalling activities that are associated with the anticancer effects of pure phytochemical compounds derived from *Aloe* extracts, aiming to provide insight into the target mechanisms of action underlying the pharmacological effects of *Aloe* components.

2. Cell death initiation by the phytochemical compounds of *Aloe*

General. Cell death is a tightly regulated process in multicellular organisms (40). There are three types of cell death: Apoptosis (type I cell death), Autophagic cell death (type II cell death) and necrosis (type III cell death) (41-43). Apoptosis is known as a programmed cell death and dysregulation of apoptosis may lead to various health conditions, e.g. potentiation of apoptosis may be associated with neurodegenerative or autoimmune diseases, while suppression of apoptosis may lead to cancer (44). Therefore, one of the most widely studied anticancer strategies is targeting the apoptotic cascade (45). Since the third form of cell death, necrosis, is characterized by the rapid loss of plasma membrane integrity and spillage of its intracellular contents (43), the tiggering of necrosis is not a valuable strategy for cancer treatments, which is why it is not discussed further. The influence of the pure phytochemical compounds from *Aloe* extracts on cell death-associated pathways are discussed in this section.

Intrinsic pathway of caspase-3 dependent apoptosis initiation. The intrinsic or mitochondrial apoptosis pathway is triggered by mitochondrial outer membrane permeabilisation and the loss of the mitochondrial membrane potential ($\Delta\Psi m$), which is in turn mediated by the oligomerisation of Bcl-2-like protein 4 (BAX) or Bcl-2 associated agonist of cell death. Reactive oxygen species (ROS) may also cause mitochondrial membrane depolarisation and the opening of BAX/Bcl-2 homologous antagonist killer (BAK) channels (46). After membrane disruption, cytochrome *c* is released from mitochondria into the cytoplasm, which binds with the apoptotic protease activating factor 1 (Apaf-1) protein to form the apoptosome and activate the caspase cascade, resulting in apoptosis (44). Mitochondrial outer membrane permeabilisation is antagonised by the anti-apoptotic Bcl-2-family of proteins, which under physiological conditions are bound to the BAX/BAK channels to inhibit their function (41). Following caspase cascade activation, cleavage of poly(ADP-ribose) polymerase (PARP-1) by caspases is considered to be the hallmark of apoptosis (47,48).

A summary of the effects of selected *Aloe* compounds on intrinsic apoptosis activation on different types of cancer is presented in Table I and Fig. S1. Pure compounds extracted from *Aloe* potently activated the intrinsic apoptotic pathway of cancer cells. However, the effect of crude *Aloe* extracts has not been studied extensively. The lyophilised *A. vera* extract from the Coral Vegetable (Miyakojima, Japan) has been reported to possess the ability to reduce BCL2 gene expression in the human hepatoblastoma HepG2 cell line (49). By contrast, treatment of the human neuroblastoma cell line IMR-32 with *A. vera* protein extract did not result in any differences in the relative expression levels of BAX, BCL2 and the cell survival-related gene myeloid cell leukaemia 1 (50), suggesting that the active components that exerted effects on these genes were unlikely to be of protein origin. Supporting this, crude *A. arborescens* whole-leaf extract was unable to induce the cleavage of PARP-1 or induce apoptosis according to the results from a Annexin V assay in spontaneously-immortalised HaCaT human skin keratinocytes, which are not cancerous, further suggesting that *Aloe* treatment did not trigger apoptosis (51).

Aloe-emodin has been indicated to induce apoptosis by upregulating the protein expression of BAX and caspases-9 and -3, whilst decreasing that of Bcl-2, thereby inducing the loss of $\Delta\Psi m$, cytochrome *c* release into the cytosol and increasing the levels of ROS. These effects were demonstrated in the human breast cancer cell lines MCF10AT and MCF7 (52), the human pancreatic adenocarcinoma cell line MIAPaCa-2 (53), the human colorectal cancer cell lines SW620 and HT29 (54), the human oral squamous cell carcinoma cell line SCC15 (55), the human stomach cancer cell line AGS (56) and in human non-small cell lung cancer (57). Immunohistochemical staining for caspase-3 in sections of xenograft tumours formed by U87MG cells in CD1 nude mice was evidently stronger in tumour tissues from mice treated with aloe-emodin (58). Furthermore, reduction of PARP-1 (116 kDa) and activation of lamin A was detected in U87MG human glioblastoma cell line treated with aloe-emodin (58). In addition, aloe-emodin has been reported to decrease the apoptosis rate, ROS production and $\Delta\Psi m$ in H_2O_2-treated neonatal rat ventricular cardiomyocytes but did not confer any effect on cardiomyocytes that were not exposed to H_2O_2, providing an example of the safety of *Aloe* compounds for non-cancerous cells (59).

Table I. Effects of phytochemical compounds from *Aloe* species on signalling networks in cancer models.

A, Apoptosis: Intrinsic pathway of apoptosis initiation, caspase-3 dependent			
Compound	Model	Target	(Refs.)
Lyophilized *A. vera* extract	Human hepatoblastoma HepG2 cell line	↓ BCL2 gene expression	(49)
A. vera protein extract	Human neuroblastoma cell line IMR-32 cell line	no differences in BAX, BCL2 and MCL1 mRNA expression	(50)
Aloe-emodin	Human breast cancer MCF-10AT and MCF-7 cell lines	↓ Bcl-2, ↓ Bcl-xL, ↑ BAX	(52)
	Human glioblastoma U87MG cell line	↓ of the non-cleaved form of PARP-1, activation of lamin A	(58)
	U87MG xenograft tumours in CD1 nude mice	Activation of caspase 3	(58)
	Human pancreatic adenocarcinoma MIAPaCa-2 cell line	DNA fragmentation and ↓ ΔΨm	(53)
	Human colorectal cancer cells SW620 and HT29	↓ Bcl-2, ↑ BAX, ↑ ROS	(54)
	Human oral squamous cell carcinoma SCC15 cell line	↑ caspase-9, ↑ caspase-3	(55)
	Human lung adenocarcinoma A549 and NCI-H1299 cell lines	↑ caspase-9, ↑ caspase-3, ↑ cleaved caspase-3, ↑ cleaved caspase-9, ↑ cleaved PARP	(57)
Gamma-irradiated aloe-emodin	Human stomach cancer AGS cell line	↑ BAX, ↑ caspase-9, ↑ caspase-3, ↑ cytosolic cytochrome c release, ↑ ROS, ↑ caspase-8-mediated BID and PARP-1 cleavage, ↓ ΔΨm, ↓ Bcl-2	(56)
Emodin	Human cervical cancer HeLa cell line	↑ cytochrome c, ↑ Apaf-1, ↓ pro-caspase-9, ↓ pro-caspase-3	(60)
	HeLa	↑ cleavage of pro-caspases, ↑ the ratio of BAX to Bcl-2 proteins	(61)
	Human hepatocarcinoma SMMC-7721 cell line	↑ cleaved caspase-3, ↑ cleaved caspase-9	(62)
	HepG2	↑ cyclophilin D, ↓ ΔΨm, ↓ ATP production, ↑ oxygen anion (O_2^-) generation in mitochondria	(63)
	Human hepatocarcinoma HepaRG cell line	↑ ROS, ↓ ΔΨm, ↑ cytochrome c release from mitochondria, ↑ BAX, ↑ cleaved caspase-3, ↑ cleaved caspase-9, ↑ cleaved PARP, ↓ Bcl-2	(63)
	Human pancreatic cancer SW1990 cell line	↓ Bcl-2, ↑ BAX, ↑ cytochrome c (cytosol), ↑ caspase-9, ↑ caspase-3	(65)
	SW1990-inoculated nude mice	↓ Bcl-2 to BAX ratio, ↑ caspase-9, ↑ caspase-3, ↑ cytochrome c release	(70)
	Human lung adenocarcinoma cells A549	↑ cytochrome c, ↑ caspase-3, ↑ caspase-9, ↑ generation of ROS, ↓ ΔΨm, ↓ Bcl-2, ↑ BAX	(66)
	A549	↑ BAX, ↓ Survivin, ↑ cytosolic cytochrome c, ↓ ΔΨm, ↑ ROS generation	(67)
	IMR-32	↑ ROS, ↑ cytoplasmic free calcium (Ca^{2+}) and NO levels, ↓ ΔΨm, ↑ caspases-9, ↑ caspase-3	(68)
	Human colorectal carcinoma SW480 and SW620 cell lines	↑ ROS	(69)

Table I. Continued.

A, Apoptosis: Intrinsic pathway of apoptosis initiation, caspase-3 dependent

Compound	Model	Target	(Refs.)
Chrysophanol	Human breast cancer MCF-7 and MDA-MB-231 cell lines	↑ cleaved caspase 3, ↑ cleaved PARP-1, ↓ Bcl-2	(71)
	Human breast cancer BT-474 and MCF-7 cell lines	↓ ΔΨm, ↑ cytosolic Ca^{2+}, ↑ BAX, ↑ cytochrome c expression, ↑ ROS	(72)
	A549	↑ ROS, ↓ ΔΨm, ↑ cytochrome c, ↑ DNA damage, did not alter the total protein levels of PARP, caspase-3, Apaf-1 and AIF	(75)
	Human small (H738) and non-small cell lung cancer (A549) cell lines	↑ BAX, ↑ caspase-3, ↑ caspase-9, ↑ PARP-1, ↓ Bcl-2, ↓ ΔΨm	(72)
	Human hepatocellular carcinoma J5 cell line	↑ ROS, ↑ cytosolic Ca^{2+} level, ↑ DNA damage, ↓ ΔΨm	(74)
	Human choriocarcinoma JEG-3 cell line	↑ ROS, ↓ ΔΨm	(76)
	Human renal cell carcinoma Caki-2 cell line	↑ ROS, no changes in the protein levels of cleaved caspase-3 and cleaved PARP	(77)
Aloin	Human gastric cancer BGC-823 and HGC-27 cell lines	↓ ROS	(78)
	HGC-27	↑ cleaved PARP, ↑ cleaved caspase-3	(79)
	Human hepatocellular carcinoma HepG2 and Bel-7402	↑ cleaved caspase-3, ↑ cleaved caspase-9	(80)
	A549	↑ BAK, ↑ BAX, ↑ PUMA, ↑ NOXA, ↑ cleaved caspase-3, ↑ cleaved caspase-9, ↓ ΔΨm, ↑ release of mitochondrial Ca^{2+}	(81)
	SW620	↓ Bcl-xL	(82)
Aloesin	Human ovarian cancer SKOV3 cell line	↑ cleavage of caspase-3, ↑ cleavage of caspase-9, ↑ cleavage of and PARP-1, ↑ BAX, ↓ Bcl-2	(87)
	SKOV3 xenograft model of ovarian cancer in athymic nude mice	More apoptotic cells by detection of cleaved caspase-3 in tumour	(87)
Esculetin	Human pancreatic cancer cell lines (PANC-1, MIAPaCa-2 and AsPC-1)	↑ caspase-3, ↑ caspase-9, ↑ cleaved caspase-3, ↑ cleaved caspase-9, ↑ cytochrome c, ↓ ΔΨm, ↓ ROS	(88)
	Human prostate cancer PC-3 cell line	↑ mRNA expressions of CASP3, BAX and CYCS (cytochrome c), ↓ mRNA expressions of BCL2	(89)
	Human laryngeal cancer HEp-2 cell line	↑ ROS	(93)
	Human gastric cancer MGC-803 cell line	↓ ΔΨm, ↑ cytochrome c release, ↓ Bcl-2/BAX ratio, ↑ caspase-9, ↑ caspase-3 activity	(90)
	MGC-803 xenograft model in nude mice	↑ caspase-3, ↓ Bcl-2, ↓ Ki-67	(90)
	SMMC-7721	↓ ΔΨm, ↑ cytochrome c release, ↓ Bcl-2, ↑ BAX, ↑ caspase-9, ↑ caspase-3	(91)
Umbelliferone	Human oral carcinoma KB cell line	↑ ROS, ↓ ΔΨm	(94)
	Human renal cell carcinoma (786-O, OS-RC-2 and ACHN) cell lines	↑ BAX, ↓ Ki-67, ↓ MCM-2, ↓ Bcl-2	(92)

Table I. Continued.

A, Apoptosis: Intrinsic pathway of apoptosis initiation, caspase-3 dependent

Compound	Model	Target	(Refs.)
Isovitexin	Human liver cancer (HepG2 and SK-Hep1) cell lines	↑ caspase-3 cleavage, ↑ PARP cleavage, ↑ BAX, ↑ cytochrome c release, ↓ Bcl-2	(95)
	HepG2 tumour-bearing mice	↑ cleaved caspase-3, ↑ cleaved PARP	(95)
	HeLa	↓ Bcl-2, ↑ BAX, ↑ caspase-3	(96)

B, Apoptosis: Intrinsic pathway of apoptosis initiation, p53-dependent

Compound	Model	Target	(Refs.)
Lyophilized *A. vera* extract	HepG2	↑ P53 mRNA expression	(49)
Aloe-emodin	U87MG xenograft CD1 nude mice	↑ p53	(58)
Emodin	A549	Activated ROS-elicited ATM-p53-Bax	(67)
	IMR-32	↑ p53, ↑ p21	(68)
	HepaRG	↑ p53	(63)
Aloin	A549	↑ phosphorylation of p53 in Ser15, Thr18, Ser20 and Ser392 sites	(84)

C, Apoptosis: Extrinsic pathway of apoptosis initiation

Compound	Model	Target	(Refs.)
Aloe-emodin	U87MG xenograft tumour tissue in CD1 nude mice	↑ caspase-8	(58)
Gamma-irradiated aloe-emodin	AGS	↑ caspase-8 protein	(56)
Emodin	HeLa	↑ Fas, ↑ FasL, ↑ FADD, ↓ pro-caspase-8	(60)
	HepaRG	↑ cleaved caspase-8	(63)
Chrysophanol	Caki-2	↓ DR5, ↓ RIP1, ↓ RIP3	(77)
Esculetin	PANC-1, MIAPaCa-2 and AsPC-1	↑ caspase-8, ↑ cleaved caspase-8	(88)
	PC-3	↑ mRNA expressions of CASP8	(89)

D, Autophagy

Compound	Model	Target	(Refs.)
Aloe-emodin	MIAPaCa-2	↑ conversion of LC3-I to LC3-II	(53)
	A549 and NCI-H1299	↑ LC3-II, ↑ Beclin1	(57)
Isovitexin	HepG2 and SK-Hep1	↑ LC3, ↑ Atg3, ↑ Atg5, ↑ Beclin1	(95)
	HepG2 tumour-bearing mice	↑ LC3II level in tumour tissue	(95)

E, Cell cycle

Compound	Model	Target	(Refs.)
Crude whole-leaf *A. arborescens* extract	Human epidermoid skin carcinoma A431 cell line, MDA-MB-231 cell line, epithelial colorectal adenocarcinoma Caco-2 cell line	↑ p21WAF	(51)
	Caco-2 and MDA-MB231	↓ cyclin D1	(51)
Aloe-emodin	U87MG	Cell cycle arrest in S andG2/M phases, ↑ p53, ↑ p21, ↓ CDK2	(58)

Table I. Continued.

E, Cell cycle

Compound	Model	Target	(Refs.)
	MIAPaCa-2	↑ fraction of cells in sub-G1 phase	(53)
	EGF-induced transformed JB6 CI 41-5a (mouse skin epidermis) cells	G1 phase arrest, ↓ cyclin D1	(110)
	MCF7	↓ cyclin D1	(111)
Gamma-irradiated aloe-emodin	AGS	↑ sub-G1 peak	(56)
Emodin	MCF7	↓ cyclin D1	(111)
	IMR-32	↑ p21	(68)
	HepaRG	S and G2/M phases arrest, ↑ p21, ↑ cyclin E, ↓ cyclin A, ↓ CDK2	(63)
	SW480 and SW620 cells	decrease in the expression of mRNA as well as the protein of Myc and cyclin D1	(69)
	Human cervical cancer HeLa and SiHa cell lines	↓ p15, ↓ p16, ↓ p27, ↓ CDK6, ↓ cyclin D1, ↓ p21, ↓ Pin1	(61)
Chrysophanol	A549	S-phase arrest, ↓ cyclin D, ↓ CDK2, ↓ thymidylate synthase	(75)
	J5	S phase arrest	(72)
	MCF-7 and MDA-MB-231	cell cycle arrest at the G1-S cell cycle checkpoint, ↓ cyclin D1, ↓ cyclin E, ↑ p21	(71)
Aloin	BGC-823 and HGC-27	S and G2 phase arrest, ↓ cyclin D1, unchanged cyclin E1	(78)
Aloesin	SKOV3	↓ cyclin A, ↓ CDK2, ↓ cyclin D1	(87)
	SK-HEP-1	↑ cyclin E, ↑ CDK2, ↑ CDC25A, ↑ enzyme activity of cyclin E/CDK2	(112)
Esculetin	PANC-1, MIAPaCa-2 and AsPC-1	↑ percentage of cells in G1 phase with ↓ percentage of cells in S and G2/M phases	(88)
	SMMC-7721	↓ c-Myc, ↓ cyclin D1	(113)
	Human prostate cancer (PC-3, DU145 and LNCaP) cell lines	G1-phase arrest	(89)
	PC-3	↑ p53, ↑ p21Cip1, ↑ p27Kip1, ↓ CDK2, ↓ CDK4, ↓ cyclin D1	(89)
Umbelliferone	KB	G0/G1 phase arrest	(94)
	786-O, OS-RC-2 and ACHN	G1 phase arrest, ↓ Ki67, ↓ MCM2, ↓ CDK2, ↓ cyclinE1, ↓ CDK4, ↓ cyclinD1	(92)

F, PI3K-AKT signalling

Compound	Model	Target	(Refs.)
Aloe-emodin	U87MG cells	↓ phosphorylation of AKT serine (p-AKT)	(58)
	JB6 CI 41-5a cell neoplastic model	↓ EGF-induced phosphorylation of MSK1, PDK1 and AKT	(110)
	A549 and NCI-H1299	↓ p-AKT, ↓ p-mTOR	(57)
Emodin	SMMC-7721	↓ p-AKT, total AKT was unchanged	(62)
	SW1990-inoculated nude mice	↓ p-AKT (Ser473) in tumour tissues	(70)
	A549	↓ p-AKT	(66)

Table I. Continued.

F, PI3K-AKT signalling

Compound	Model	Target	(Refs.)
	U87MG	↓ phosphorylation of AKT/PKB	(120)
	U87MG bearing mice	↓ p-AKT	(120)
Chrysophanol	BT-474 and MCF-7	↓ p- AKT, ↓ p-P70S6K, ↓ p-S6K	(73)
	HCT116 and SW480	↓ p-PI3K, ↓ p-AKT, total AKT and PI3K were unchanged	(121)
	Human colon carcinoma SNU-C5 cell line	↓ EGF-induced activation of EGFR, AKT, mTOR, p70S6K	(122)
	JEG-3	↑ p-AKT, ↑ p-P70S6K	(76)
Aloin	BGC-823 and HGC-27	↓ phosphorylation and activation of Src, AKT, mTOR, P70S6K and S6K	(78)
	HepG2 and Bel-7402	↓ p-PI3K, ↓ p-AKT, ↓ p-mTOR	(80)
	HepG2 orthotopic xenograft tumours in BALB/c athymic nude mice	↓ p-PI3K, ↓ p-AKT, ↓ p-mTOR	(80)
	HGC-27 and rhHMGB1- induced HGC-27 cells	↓ p-AKT, ↓ p-mTOR, ↓ p-P70S6K, ↓ p-S6K, ↓ p-4EBP1	(79)
Esculetin	PC-3	↑ PTEN, ↓ p-AKT	(89)
	MGC-803	↓ IGF-1, ↓ p-PI3K, ↓ p-AKT	(90)
	MGC-803 xenograft nude mice	↓ IGF-1, ↓ p-PI3K, ↓ p-AKT, total PI3K and AKT were unchanged	(90)
	SMMC-7721	↓ IGF-1, ↓ p-PI3K, ↓ p-AKT	(91)
Umbelliferone	786-O, OS-RC-2 and ACHN	↓ p110γ	(92)

G, JAK-STAT signalling

Compound	Model	Target	(Refs.)
Aloin	BGC-823 and HGC-27	↓ p-STAT3	(92)
Esculetin	HEp-2 *in vitro* and in HEp-2 xenograft tumours in nude mice *in vivo*	↓ p-JAK1, ↓ p-JAK2, ↓ p-STAT3	(93)
Aloe polysaccharides	HT-29 *in vitro* and 2,4,6-trinitrobenzenesulfonic acid-induced colitis in Sprague Dawley rats *in vivo*	↓ JAK2, ↓ p-JAK2, ↓ STAT-3, ↓p-STAT3	(129)

H, MAPK-ERK signalling

Compound	Model	Target	(Refs.)
Emodin	SMMC-7721	↑ p-ERK, total ERK was unchanged	(62)
	HepG2	↓ p-ERK	(64)
	A549	↓ p-ERK	(66)
	U87MG	↓ p-ERK1/2	(120)
	U87MG bearing mice	↓ p-ERK1/2	(120)
Chrysophanol	Modelled benign prostatic hyperplasia in rats and in RWPE-1 cell line	↓ p-ERK	(105)
	BT-474 and MCF-7	↓ p-ERK1/2	(73)
	JEG-3	↑ p-ERK1/2, ↑ p-P90RSK	(76)
	SNU-C5	↓ EGF-induced activation of ERK1/2	(122)
Aloin	HGC-27 and rhHMGB1-induced HGC-27	↓ p-ERK, ↓ p-P90RSK, ↓p-CREB	(79)
Aloesin	SKOV3	↓ p-ERK, ↓ p-MEK	(87)

Table I. Continued.

I, MAPK-JNK/p38 signalling

Compound	Model	Target	(Refs.)
Emodin	SMMC-7721	↑ p-p38, ↓ p-JNK	(62)
Chrysophanol	BT-474	↓ p-p38, ↓ p-JNK	(73)
	MCF-7	↑ p-p38, ↑ p-JNK	(73)
Aloin	A549	↑ p-c-Jun, ↑ p-p38	(81)
Aloesin	SKOV3	↓ p- JNK, ↓ p-p38	(87)

J, TGF-β signalling

Compound	Model	Target	(Refs.)
Emodin	HeLa and SiHa	↓ TGF-β receptor II	(61)
	HeLa	↓ SMAD4, ↓ p- SMAD2, ↓ p-SMAD3	(61)

K, Wnt signalling

Compound	Model	Target	(Refs.)
Aloe-emodin	JB6 CI 41-5a cell neoplastic model	↓ EGF-induced phosphorylation of GSK3β	(110)
Emodin	HeLa	↓ β-catenin, ↓ p-GSK-3β (ser9), ↓ total GSK-3β	(61)
	SW480 and SW620	↓ β-catenin, ↓ TCF-4)	(69)
Esculetin	SMMC-7721	↓ β-catenin, ↑ p-β-catenin (Ser33/Ser37/Thr41)	(113)
Aloesin	Super TCF NIH/3T3 (transfected with a Super TCF reporter gene plasmid)	↑ mRNA expression of Ccnd1, Ctnnb1 and Axin2	(153)
	Human colon cancer HCT-15 cell line	↑ mRNA expression of c-MYC, MMP-7, DKK1, AXIN2 and APC	(153)
	Human colon cancer RKO cell line	↑ mRNA expression of c-MYC, CCND1, GSK-3β, AXIN2 and APC	(153)

L, Immunomodulation (TNF-α- NF-κB pathway) in cancer models

Compound	Model	Target	(Refs.)
Emodin	SW1990 and PANC-1	↓ NF-κB	(65,70,104)
	Flagellin-stimulated HT-29	↓ TLR5, ↓ MyD88, ↑ IκB, ↓ nuclear translocation of NF-κB p65, ↓ release of IL-8	(172)
	U87MG hyaluronic acid-induced *in vitro* invasion model	↓ NF-κB	(120)
Chrysophanol	MCF-7 and MDA-MB-231	↓ p-IκB, ↓p-p65 NF-κB	(74)
Aloin	BGC-823 and HGC-27	↓ p-IκBα, ↓ p-NFκB	(78)
	Dimethylhydrazine-induced colon carcinogenesis in Wistar rats	↓ oxidative stress enzymes (glutathione peroxidase, glutathione-S-transferase, glutathione reductase, reduced glutathione), ↓ cyclooxygenase-2, ↓ NOS,↓ IL-6, ↓ PCNA, ↓ TNF-α release	(173)

Table I. Continued.

L, Immunomodulation (TNF-α- NF-κB pathway) in cancer models			
Compound	Model	Target	(Refs.)
Esculetin	PANC-1, MIAPaCa-2 and AsPC-1	↓ NF-κB, IκB was unchanged	(88)
	PC-3	↑ mRNA expression of TNFR1	(89)

↑, stimulation or activation; ↓, suppression or inhibition; ΔΨm, outer mitochondrial membrane potential; ATM, ataxia telangiectasia mutated kinase; BAD, Bcl-2 associated agonist of cell death; BAX, Bcl-2-like protein 4; CDK, cyclin-dependent kinases; DR, death receptor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FADD, Fas-associated protein with death domain; IκB, inhibitor of κB; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinases; MCM2, minichromosome maintenance complex component 2; MEK, mitogen-activated ERK kinase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; NO, nitric oxide; NOS, NO synthase; p-AKT, phosphorylated AKT; PARP, poly(ADP-ribose)polymerase; PI3K, phosphoinositide 3-kinase; RIP1, receptor interacting protein 1; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor β.

Emodin has been previously indicated to activate the intrinsic mitochondrial pathway of apoptosis at all key stages in the human cervical cancer cell line HeLa (60,61), the human hepatocarcinoma cell lines SMMC-7721 (62) and HepaRG (63), HepG2 (64) the human pancreatic cancer cell line SW1990 and its corresponding gemcitabine-resistant variant SW1990/Gem (65), the human lung adenocarcinoma cell line A549 (66,67), the human neuroblastoma cell line IMR-32 (68) and the human colorectal carcinoma cell lines SW480 and SW620 (69). In addition, emodin treatment induced upregulation of Apaf-1 (60) and cyclophilin D expression (64) and reduced that of Survivin (67). It was indicated in tumour tissues from SW1990-inoculated nude mice that treatment with either emodin alone or emodin combined with gemcitabine reduced the Bcl-2/BAX ratio, while increasing caspase-9 and caspase-3 activation and cytochrome *c* release (70).

The anthraquinone chrysophanol also possess stimulatory effects on the intrinsic apoptotic pathway, including the suppression of Bcl-2 expression (71,72) and the upregulation of pro-apoptotic proteins, including BAX (71,72), caspase-3 (71,72), caspase-9 (72) and PARP-1 (71,72). In addition, chrysophanol has also been reported to induce the loss of ΔΨm (72-76) and to increase cytosolic Ca^{2+} concentrations (73,74), cytochrome *c* release (73,75) and ROS generation (73,75-77). However, other studies reported that chrysophanol did not exert any effects on the protein expression levels of cleaved caspase-3 (75,77), cleaved PARP (77), total protein levels of PARP (75), Apaf (75) or apoptosis-inducing factor (75). It was hypothesised that the reason for the caspase cascade not being activated despite apoptosis being initiated after chrysophanol treatment was due to a reduction in total ATP, meaning that cytochrome *c* was not able to interact with Apaf-1 and pro-caspase-9 to form the apoptosome (71).

Aloin was able to markedly reduce the production of ROS in the human gastric cancer cell lines BGC-823 and HGC-27 (78), whilst increasing the cleavage of PARP and caspase-3 in a dose-dependent manner in HGC-27 cells (79). The protein levels of cleaved caspase-3 and caspase-9 were reported to be significantly increased in the human liver cancer cell lines HepG2 and Bel-7402 following aloin treatment (80). Aloin also significantly increased the protein expression levels of the intrinsic apoptosis pathway effectors BAK, BAX, p53 upregulated modulator of apoptosis, phorbol-12-myristate-13-acetate-induced protein 1, cleaved caspase-3 and caspase-9, which resulted in the loss of ΔΨm and release of mitochondrial Ca^{2+} in A549 cells (81). The anti-apoptotic Bcl-xL gene was significantly reduced in response to aloin treatment of SW620 cells (82). Furthermore, aloin did not have any adverse effects on the mouse macrophage RAW264.7 cell line (83,84), but lipopolysaccharide (LPS)-induced cleavage of caspase-9 and caspase-3 in RAW 264.7 cells was largely suppressed by aloin (85). Aloin was also documented to reduce hypoxia/reoxygenation-induced apoptosis of primary mouse hepatocytes *in vitro* by suppressing BAX and cleaved caspase-3 protein levels, whilst increasing Bcl-2 expression (86), indicating that the aforementioned apoptotic effects were only selective on cancer cells.

Aloesin treatment of the ovarian cancer cell line SKOV3 led to the increased proteolytic cleavage of caspase-3, caspase-9 and PARP-1, as well as increased expression of BAX protein whilst decreasing that of Bcl-2 protein in a dose-dependent manner (87). In addition, cleavage of caspase-3 was stimulated by aloesin treatment in an ovarian cancer SKOV3 cell-derived xenograft model in athymic nude mice (87).

Treatment with esculetin reduced the level of ΔΨm, increased cytochrome *c* release from mitochondria into the cytoplasm and reduced the Bcl-2/BAX ratio in addition to increasing caspase-9 and caspase-3 activity in different cancer models *in vitro* (88-91). The esculetin treatment group in an MGC-803 xenograft nude mouse model exhibited increased caspase-3 expression, as well as reduced Bcl-2 and nuclear Ki-67 expression *in vivo* (90).

Umbelliferone effectively upregulated the expression of BAX whilst downregulating that of Ki-67, minichromosome maintenance complex component 2 (MCM-2) and Bcl-2 proteins in three human renal cell carcinoma cell lines, 786-O, OS-RC-2 and ACHN (92). In addition, umbelliferone has also been indicated to enhance ROS production in the human laryngeal cancer cell line HEp-2 (93) and to reduce the ΔΨm in the human oral carcinoma cell line KB (94).

Treatment of the human liver cancer cell lines HepG2 and SK-Hep1 with isovitexin resulted in a dose-dependent cleavage of caspase-3 and PARP, an increase in BAX expression and cytochrome *c* release into the cytoplasm, in addition to reducing Bcl-2 expression (95). Isovitexin also triggered HeLa cell death through an oxidative stress-mediated signalling pathway and downregulating the protein expression of Bcl-2, whilst upregulating that of BAX and caspase-3 (96). In addition, isovitexin has been reported to increase caspase-3 and PARP cleavage in HepG2 cell tumour-bearing mice *in vivo* (95). By contrast, isovitexin was able to protect against LPS-induced oxidative damage by suppressing intracellular ROS generation in RAW 264.7 cells (97).

Intrinsic p53-dependent pathway of apoptosis initiation. The tumour suppressor p53 is able to induce various types of cell death, senescence, cell cycle arrest or subsequent DNA repair depending on the level of cellular damage. Mouse double minute 2 homolog (MDM2) constitutively binds to p53, leading to the ubiquitination and degradation of p53 when it is not required. DNA damage activates the ataxia telangiectasia mutated (ATM)/ataxia telangiectasia and Rad3-related kinases, which are able to phosphorylate and activate checkpoint kinase 1 (Chk1). Chk1 then phosphorylates p53 to disrupt MDM2 binding. p53 in turn upregulates the transcription of the cyclin-dependent kinase (CDK) inhibitor p21, induces the formation of a complex consisting of p53-induced death domain protein (PIDD1) and the receptor interacting protein (RIP)-associated ICH-1, CED-3-homologous protein with death domain, known as the PIDDosome, to activate caspase-2. In addition, p53 interacts with the Bcl-2 family of proteins and mitochondrial membranes to induce mitochondrial outer membrane permeabilisation and triggers the mitochondrial pathway of apoptosis (98).

All previous findings on the stimulation of p53 expression by *Aloe* compounds are listed in Table I and summarised in Fig. S1. A lyophilised *A. vera* extract was indicated to increase p53 mRNA expression in HepG2 cells in a time- and dose-dependent manner (49). In another study, aloe-emodin treatment induced p53 protein expression in tumour tissues from CD1 nude mice injected with U87MG cells (58). Emodin also induced apoptosis by activating the ROS-elicited ATM/p53/BAX signalling pathway in A549 cells (67). In addition, it was reported that emodin upregulated the p53 protein and phosphorylation of ATM, but did not alter the level of total ATM protein expression (67). Emodin also led to a direct increase in p53 and p21 protein expression in IMR-32 cells (68) and upregulated p53 expression in HepaRG cells (63), whereas aloin activated p53 phosphorylation on the Ser15, Thr18, Ser20 and Ser392 residues in A549 cells (81).

Extrinsic pathway of apoptosis initiation. The extrinsic pathway of apoptosis initiation via the death receptor (DR) signalling pathway is triggered by the activation of the death receptor family, which includes tumour necrosis factor (TNF) receptor 1 (TNFR1), Fas/CD95, DR3, DR4 and DR5, which results in the assembly of a caspase-activation platform called the death-inducing signalling complex. This platform recruits and activates caspase-8 via the adaptor protein Fas-associated protein with death domain (FADD), which engages in the extrinsic apoptotic pathway (41).

Information available regarding the influence of *Aloe*-derived compounds on the extrinsic apoptosis pathway remains scarce (Table I). Aloe-emodin has been indicated to activate caspase-8 in U87MG xenograft tumour tissues in CD1 nude mice (58), whereas γ-irradiated aloe-emodin increased caspase-8 protein expression in AGS cells (56).

Emodin treatment of HeLa cells led to upregulation of Fas, Fas ligand and FADD expression and simultaneously downregulated pro-caspase-8 in a dose-dependent manner at both the mRNA and protein levels (60). Furthermore, the same treatment of HepaRG cells led to upregulation of cleaved caspase-8 levels (63).

The expression levels of DR5, RIP1 and RIP3 were revealed to be lower following treatment with chrysophanol compared with those in the untreated control in the human renal cell carcinoma cell line Caki-2 (77). Since simultaneous RIP1 and RIP3 expression allows normal cells to escape apoptosis and survive (41), chrysophanol may selectively prevent the survival of cancer cells. However, aloin treatment failed to induce activation of the extrinsic apoptosis pathway, as it did not affect the levels of cleaved caspase-8 and -10 and CD95 in A549 cells (81). Esculetin was reported to increase the expression of caspase-8 and cleaved caspase-8 in PANC-1, MIAPaCa-2 and AsPC-1 cells (88) and in another study, it increased the mRNA expression levels of caspase-8 in PC-3 cells (89).

Autophagy. Limited information is available on the effect of *Aloe* compounds on the autophagy process in cancer cells. However, aloe-emodin induced autophagy in MIAPaCa-2 cells by enhancing the conversion of microtubule-associated proteins 1A/1B light chain 3B (LC3)-I to LC3-II in a dose-dependent manner (53). In addition, aloe-emodin treatment increased the number of endogenous LC3 puncta, LC3B-II and Beclin1 expression in A549 and NCI-H1299 cells (57). Isovitexin also induced autophagy in liver cancer cell lines, HepG2 and SK-Hep1, activating LC3, autophagy related (Atg)3, Atg5 and Beclin1 in a dose-dependent manner. Furthermore, isovitexin increased LC3II levels in tumour tissues from HepG2 cell tumour-bearing mice *in vivo* (95).

3. Anticancer selectivity

In terms of safety, investigation of the potential adverse effects of *Aloe* compounds on normal cells is of high importance, i.e. their associated cytotoxic effects should be selective for cancer cells. In previous studies, high selectivity and low toxicity of *Aloe* phytochemical compounds against normal cells have been reported.

Treatment with an aqueous whole-leaf extract of *A. vera* was indicated to selectively induce cell death in PC-3 cells but not in the normal human chondrocyte cell line C28/I2 (99). The IC_{50} value of the *A. vera* leaf extract on the MCF-7 cell line was 23 μg/ml, whilst that for the non-tumour mouse embryonic fibroblast cell line NIH-3T3 was 332 μg/ml (100). This selectivity was comparable with that of the standard agent tamoxifen (100).

Aloe polysaccharides (Chinese Academy of Sciences, Shanghai Institute of Materia Medica) elicited no significant cytotoxicity on the Madin-Darby canine kidney cell line at concentrations ranging from 20 to 640 μg/ml (31) and neither did *Aloe* polysaccharides on the cell viability of HaCaT cells following treatment at concentrations of 20, 40 and 80 μg/ml (101).

Aloe-emodin was reported to specifically inhibit MCF-10AT and MCF-7 cell growth, but did not exert any toxicity against the human non-tumorigenic spontaneously immortalised breast epithelial cell line MCF-10A (52). Furthermore, administration of aloe-emodin did not result in any cytotoxicity towards human peripheral mononuclear cells at all concentrations tested in the 0.001-100 μM range, whilst the IC_{50} values of aloe-emodin towards the lymphoblastic leukaemia cell line CCRF-CEM, the human colorectal carcinoma cell line HCT116, U87MG, the human breast adenocarcinoma MDA-MB-231 cell line and the tumorigenic human cell line 293T were in the 9.8-22.3 μM range (102). IC_{50} values were calculated to be in the range of 11.1-33.76 μM for the drug-resistant cell lines CEM/ADR5000, HCT116($p53^{-/-}$), U87MGΔEGFR, MDA-MB- 231-BCRP clone 23 and HEK293-ABCB5 (102). Aloe-emodin did not confer any cytotoxic effects on neonatal rat ventricular myocytes at the concentration of 100 μM (59). One approach to studying the potential anticancer and radio-protective effects of compounds is the measurement of radiolytic products following γ-irradiation (56). It was indicated that γ-irradiated aloe-emodin demonstrated increased anticancer activity in Moloney sarcoma virus-transformed BALB/3T3 mouse embryo fibroblast cells or in B16BL6 mouse skin melanoma cells, MCF-7 or THP-1 human monocytic leukaemia cells, but did not induce cytotoxicity in normal mouse embryonic fibroblast cells (BALB/3T3 clone A31) (56).

Emodin was reported to induce cytotoxicity in SW480 and SW620 cells, but not to a similar extent in normal human colon CCD 841 CoN (American Type Culture Collection® CRL-1790™) cells (69). Emodin concentrations of ≤120 μg/ml did not exert any toxic effects in normal primary mouse fibroblasts (103). Emodin, gemcitabine and their combination provided high selectivity against SW1990 and PANC-1 cells compared with that in HPNE E6/E7 human pancreatic normal epithelial cells (104). A unique feature of emodin appeared to be the induction of cell death in microvascular endothelial cells in implanted pancreatic cancer tissues, whilst gemcitabine did not have any effect on pancreatic cancer-derived endothelial cells (104).

Chrysophanol did not affect the viability of the normal human prostatic epithelial cell line RWPE-1 at concentrations ≤5 μM, but was effective against benign prostatic hyperplasia cells (105). Furthermore, chrysophanol demonstrated selective activity against J5 cells with no toxicity against A10 normal rat myoblast cells (74). Furthermore, chrysophanol did not exert any cytotoxicity against MCF-12A normal human breast cells at concentrations of ≤100 μM, but did exhibit anticancer activity against BT-474 and MCF-7 cells (73).

Aloin at concentrations ≤1,000 μM did not affect the viability of human umbilical cord vascular endothelial cells (HUVECs) (106,107) and RAW264.7 macrophages were not affected by the aloin (barbaloin) at ≤500 μM (200 μg/ml) (83-85).

Esculetin treatment at ≤1,700 μM did not result in cell death of GES-1 normal human gastric epithelial cells, whilst anticancer activity against MGC-803, BGC-823 and HGC-27 cells was observed from a concentration of 560 μM (90).

4. Cell cycle regulation

Cyclins are key regulators of the cell cycle that undergo a cycle of synthesis and degradation during each cell cycle, whereas the expression of cyclin-dependent kinases (CDK) remains constant but is activated in the presence of the appropriate regulatory cyclins. The levels of cyclin-CDK complexes, including D-CDK4/6, E-CDK2, A-CDK1/2 and B-CDK1, are essential for cell cycle progression (108,109).

Treatment with crude whole-leaf *A. arborescens* extract induced p21 protein expression in the human epidermoid skin carcinoma cell line A431, as well as in MDA-MB-231 cells and the epithelial colorectal adenocarcinoma cell line Caco-2, whereas cyclin D1 expression was supressed in Caco-2 and MDA-MB-231 cells (51). The cell-cycle distribution was not affected in HaCaT cells after 48 h of treatment with the *A. arborescens* crude whole-leaf extract (51). However, proteomic analysis of HaCaT cells after exposure to *A. arborescens* whole-leaf extract revealed the expression of anti-proliferative (78-kDa glucose-regulated protein 78, prohibitin and stathmin) and antimicrobial (dermcidin) proteins (51).

Aloe-emodin treatment of U87MG cells resulted in cell cycle arrest at the S and G2/M phases by increasing the expression of p53, p21 and reducing that of CDK2 (58). In another study, aloe-emodin administration led to an increase in the fraction of cells in the sub-G1 phase in MIAPaCa-2 cells (53). Aloe-emodin also induced cell cycle arrest at the G1 phase by reducing cyclin D1 transcription in EGF-induced transformed mouse skin epidermis cell line JB6 CI 41-5a in a dose-dependent manner (110). In addition, γ-irradiated aloe-emodin increased the population of cells in sub-G1 phase in a dose-dependent manner in AGS cells (56).

Cyclin D1 expression was decreased after treatment with either emodin or aloe-emodin in MCF7 cells (111). Treatment of IMR-32 cells with emodin led to a direct increase in p21 protein expression (68). Emodin treatment of HepaRG cells blocked cell cycle progression at the S and G2/M phases by upregulating p21 and cyclin E, whilst downregulating cyclin A and CDK2 (63). Emodin also led to a reduction in mRNA and protein expression of c-Myc and cyclin-D1 in SW480 and SW620 cells (69). However, contradictory effects were observed in the human cervical cancer cell lines HeLa and SiHa, where emodin blocked cell cycle progression, but simultaneously inhibited the expression of p15, p16, $p27^{Kip1}$, CDK6, cyclin D1, p21 and peptidylprolyl cis/trans isomerase, NIMA-interacting 1 (61).

Chrysophanol was reported to induce S-phase arrest in A549 cells by decreasing the expression of cyclin D, CDK2 and thymidylate synthase protein (75). J5 cells exposed to chrysophanol also displayed S-phase arrest and necrotic cell death (74). Following chrysophanol treatment of MCF-7 and MDA-MB-231 cells, cell cycle arrest at the G1/S checkpoint

occurred by inhibition of cyclin D1 and E expression and upregulation of p21 levels (71).

Aloin caused cell cycle arrest at the S and G2 phases in both BGC-823 and HGC-27 cells by reducing cyclin D1 protein expression, but did not alter the expression levels of cyclin E1 (78).

The levels of the S-G2/M-related proteins cyclin A, CDK2 and cyclin D1 were downregulated in SKOV3 cells following aloesin treatment in a dose-dependent manner (87). Treatment with aloesin resulted in increased levels of cyclin E, CDK2 and CDC25A in SK-HEP-1 cells (112).

Esculetin treatment significantly increased the percentage of PANC-1, MIAPaCa-2 and AsPC-1 cells in G1 phase with a concomitant reduction in the percentage of cells in the S and G2/M phases (88). Treatment of SMMC-7721 cells resulted in a significant reduction in the mRNA and protein levels of c-Myc and cyclin D1 (113). Esculetin caused cell cycle arrest in G1-phase in the human prostate cancer cell lines PC-3, DU145 and LNCaP (89). This appeared to be due to the increased expression of p53, $p21^{Cip1}$ and $p27^{Kip1}$, but reduced expression of CDK2, CDK4 and cyclin D1 in PC-3 cells (89).

Treatment of KB cells with umbelliferone induced G0/G1 phase arrest of the cell cycle (94). Umbelliferone induced G1 phase arrest in 786-O, OS-RC-2 and ACHN cells by downregulating the protein expression of Ki67, minichromosome maintenance complex component 2, CDK2, cyclin E1, CDK4 and cyclin D1 (92). Acemannan significantly enhanced the proliferation of mouse skin primary fibroblasts in a dose-dependent manner *in vitro* by promoting G1- to S phase progression (114). However, neither did acemannan induce cell apoptosis nor affect cyclin D1, cyclin E or CDK2/4 protein expression (114).

All effects of *Aloe* phytochemical compounds on cancer models in terms of cell cycle regulation are summarised in Table I and Fig. S2. Aloe-derived phytochemical compounds were able to block cell cycle progression in various cancer cell lines at different phases. However, the exact molecular effect on cell cycle regulators requires further evaluation for emodin and aloesin, since opposite effects were shown on p21 (61,63,68) for emodin and on CDK2 (87,112) for aloesin, which, at the same time, did not abrogate the final biological effect as cell cycle arrest.

5. Cell signalling

PI3K/AKT pathway. The phosphoinositide 3-kinase (PI3K)/AKT pathway is a potent signalling pathway that mediates cell proliferation and survival. Growth factors or hormones are able to activate tyrosine kinase receptors, which then activates PI3K to produce phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at the plasma membrane. PIP3 then recruits inactive cytosolic AKT, 3-phosphoinositide-dependent protein kinase 1 (PDK1) and mTOR to the membrane, where PDK1 and mTORC2 phosphorylate and activate AKT (115-117). Phosphorylated (p-) AKT levels may be used as a predictive factor of cancer progression (118). The levels of p-AKT were indicated to be elevated in hepatocellular carcinoma (53%) compared with those in cirrhotic tissues (12%) and were absent in normal liver samples ($P<0.0001$). Therefore, p-AKT levels markedly differ between hepatic carcinoma and normal liver tissue (119).

After treatment with aloe-emodin, AKT phosphorylation was decreased in U87MG (58), A549 and NCI-H1299 cells (57). Aloe-emodin also inhibited EGF-induced phosphorylation of mitogen- and stress-activated kinase 1, PDK1 and AKT in a dose-dependent manner in the neoplastic JB6 CI 41-5a cell model (110).

In SMMC-7721 cells, emodin suppressed the activation of AKT, but did not decrease total AKT levels (62). Treatment with emodin in A549 cells also reduced the phosphorylation of AKT (66). In addition, pre-treatment of U87MG cells with emodin significantly decreased the phosphorylation of AKT (120). Oral administration of emodin effectively suppressed AKT phosphorylation in mice bearing U87MG tumours *in vivo* (120). However, no significant changes were observed in the ratios of p-PI3K/PI3K and p-AKT/AKT in normal primary mouse fibroblasts after emodin treatment (103). In primary mouse fibroblasts derived from hypertrophic scar tissues in C57BL/6 mice, emodin treatment markedly inhibited PI3K and AKT activity (103).

Chrysophanol inhibited the phosphorylation of AKT, p70S6K and S6K in the BT-474 and MCF-7 cell lines, whilst endoplasmic reticulum stress-regulatory proteins, including double-stranded RNA-dependent protein kinase-like endoplasmic reticulum kinase, eukaryotic translation initiation factor 2 α, inositol-requiring enzyme-1α and growth arrest and DNA damage-inducible protein 153 were activated (73). In addition, chrysophanol suppressed hypoxia-induced activation of PI3K/AKT signalling in HCT116 and SW480 cells by reducing the p-PI3K/PI3K and p-AKT/AKT ratios (121). Chrysophanol was also observed to suppress EGF-induced activation of EGFR, AKT, mTOR and p70S6K in the human colon carcinoma SNU-C5 cell line (122). However, treatment of JEG-3 cells with chrysophanol caused an increase in AKT and p70S6K phosphorylation in a dose- and time-dependent manner (76).

Aloin treatment led to downregulation of the phosphorylation and activation of Src, AKT, mTOR, p70S6K and S6K in a dose-dependent manner in BGC-823 and HGC-27 cells (78). After aloin exposure, phosphorylation of PI3K, AKT and mTOR was also significantly reduced in HepG2 and Bel-7402 cells *in vitro* and in orthotopic xenografts derived from HepG2 cells *in vivo* (80). Aloin was reported to inhibit the phosphorylation of AKT, mTOR, p70S6K, S6K and eukaryotic translation initiation factor 4E-binding protein 1 in HGC-27 and in recombinant human high-mobility group box 1 (rhHMGB1)-induced HGC-27 cells (79). However, in the non-cancerous LPS-stimulated model of RAW264.7 macrophages, aloin treatment led to downregulation of the phosphorylation levels of PI3K and AKT (84).

Esculetin markedly upregulated the expression levels of PTEN and suppressed the phosphorylation of AKT in PC-3 cells (89). The protein expression of insulin-like growth factor-1 (IGF-1), PI3K and AKT activation was reduced after esculetin treatment in SMMC-7721 cells (91). The protein expression of IGF-1 and the phosphorylation levels of PI3K and AKT were observed to be decreased after esculetin treatment in MGC-803 cells (90). Furthermore, in an MGC-803 nude mouse xenograft model, the levels of IGF-1 expression, as well as PI3K and AKT phosphorylation were decreased, whilst the expression levels of total PI3K and AKT remained unchanged following esculetin treatment (90).

Treatment with umbelliferone led to a concentration-dependent reduction in the expression of the p110γ PI3K subunit in the 786-O, OS-RC-2 and ACHN cell lines (92).

Acemannan was indicated to activate the phosphorylation of AKT, mTOR and p70S6K in primary mouse skin fibroblast cells *in vitro*, whilst activating mTOR in the wound tissue of an *in vivo* cutaneous wound model of BALB/c mice (114).

The effective suppression of the PI3K/AKT pathway mediated by the various phytochemical compounds isolated from the *Aloe* extracts and has been demonstrated (Table I and Fig. S3). However, certain controversial data regarding the effects of chrysophanol on the choriocarcinoma cell line remain, which require further investigation and verification.

Janus kinase (JAK)/ signal transducer and activator of transcription (STAT) pathway. The JAK/STAT signalling pathway is important for the regulation of angiogenesis, proliferation, migration, cell survival, haematopoiesis and development. This pathway has been demonstrated to be constantly active in malignant cells (123-125). The binding of an extracellular ligand to their respective receptors, including interleukins (ILs), vascular endothelial growth factor (VEGF) or interferons (IFN), leads to receptor oligo- or dimerisation, allowing the association and auto-transphosphorylation of intracellular JAKs. Activated JAKs then phosphorylate the intracellular domains of the receptors and STATs. Phosphorylated STATs subsequently dimerise and translocate into the nucleus, where they bind to enhancer sequences of target genes to activate transcription (126,127).

The effects of *Aloe* extracts and associated phytochemical compounds on the JAK/STAT signalling pathway in cancer models are summarised in Table I and Fig. S3. The phosphorylation of STAT3 was suppressed by aloin treatment in BGC-823 and HGC-27 cells (78). Furthermore, treatment with aloin downregulated STAT3 phosphorylation in a dose-dependent manner but did not alter total STAT3 expression in SW620 cells. In the non-cancerous cell line RAW264.7, phosphorylation of STAT1, STAT3, JAK1 and JAK2 were not affected by aloin treatment (83). In addition, aloin did not affect STAT1 phosphorylation in HUVECs (128).

The relative protein expression levels of JAK1, JAK2 and STAT3 in HEp-2 cells *in vitro* and in HEp-2 xenograft tumours in nude mice *in vivo* were not changed after treatment with esculetin (93). The phosphorylation levels of JAK1, JAK2 and STAT3, by contrast, were significantly decreased in a dose-dependent manner (93).

Aloe polysaccharides also induced the downregulation of JAK2, p-JAK2, STAT-3 and p-STAT3 protein levels in an ulcerative colitis model using the HT-29 cell line *in vitro* and in a rat model of 2,4,6-trinitrobenzenesulfonic acid-induced colitis *in vivo* (129).

Mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway. The MAPK/ERK signalling pathway regulates cellular signalling under both normal and pathological conditions. Dysregulation of this cascade results in the development of different types of cancer (130,131). Binding of growth factors or cytokines, including EGF and hepatocyte growth factor, activates the corresponding receptors, which results in the recruitment of the adaptor protein growth factor receptor-bound protein 2 and the guanine nucleotide exchange protein factor son of sevenless to activate the Ras/Raf/MAPK/ERK1/2 cascade. This is followed by activation of target gene transcription. ERK1/2 is also able to activate the ribosomal protein S6 kinase A1 (p90RSK) in the cytosol, which then enters the nucleus and activates the transcription of target genes (130,132). The MAPK/ERK signalling activation is essential for cell division, proliferation, differentiation and angiogenesis.

Treatment of proliferating HaCaT cells with crude whole-leaf extract isolated from *A. arborescens* resulted in reduction of ERK phosphorylation (51).

Emodin reduced the levels of p-ERK in HepG2 cells (64). In addition, in A549 cells, treatment with emodin resulted in decreased phosphorylation of ERK (66). Decreased levels of p-ERK1/2 after emodin treatment were also demonstrated on U87MG cells *in vitro* and in mice bearing U87MG tumours *in vivo* (120). By contrast, emodin treatment of SMMC-7721 cells increased the phosphorylation of ERK whilst not altering total ERK expression (62).

In prostate tissues of a rat model of benign prostatic hyperplasia, the upregulated phosphorylation of ERK was reversed by chrysophanol treatment, back to the levels similar to those in the corresponding normal controls (105). Chrysophanol was also reported to inhibit the phosphorylation of ERK1/2 in the BT-474 and MCF-7 cell lines (73). Chrysophanol was observed to suppress EGF-induced activation of ERK1/2 in SNU-C5 cells (122). However, treatment of JEG-3 cells with chrysophanol increased p-ERK1/2 levels after 5 min of exposure, in addition to increasing protein levels of p-p90RSK with the maximum peak reached at 5 min, which was sustained for a period of 60 min (76).

Aloin was able to suppress the phosphorylation of ERK, p90RSK and the cyclic adenosine monophosphate response element-binding protein (CREB) in HGC-27 cells and in rhHMGB1-induced HGC-27 cells (79). By contrast, phosphorylation of ERK was not affected by aloin treatment of RAW264.7 cells (83). In addition, p-ERK1/2 and RUNX family transcription factor 2 protein levels in rat and human BM-MSCs were upregulated in the presence of aloin in a dose-dependent manner (133). Aloin suppressed D-galactose (D-gal)-induced ERK phosphorylation in the hippocampus of mice, whilst the total levels of ERK remain unchanged (134). Aloin treatment also downregulated polyphosphate (PolyP)-induced activation of ERK1/2 (106), whilst significantly inhibiting transforming growth factor-β (TGF-β)-induced protein (TGFBIp)-induced activation of ERK1/2 in HUVECs (107).

Aloesin dose-dependently decreased the phosphorylation levels of ERK and MAPK in SKOV3 cells (87), whilst the opposite effect was observed in non-cancerous cells. Aloesin treatment of HaCaT and RAW264.7 cells increased ERK phosphorylation (135). Isovitexin treatment inhibited the LPS-stimulated phosphorylation of ERK1/2 in RAW264.7 cells but did not have any effect on RAW264.7 cells in the absence of LPS stimulation (97). All of the effects of *Aloe* phytochemical compounds on the MAPK/ERK signalling pathway are summarised in Table I and Fig. S3. Most of the aforementioned compounds appeared to have exerted suppressive effects on the MAPK/ERK signalling pathway, with various controversial results for certain anthraquinones,

including aloe-emodin, emodin and chrysophanol. This may be due to cancer-type specificity or inconsistent drug concentrations used, which require further verification. Furthermore, other MAPK signalling pathways, including JNK and p38, may also engage in crosstalk with the ERK signalling cascade, making it difficult to draw definitive conclusions on the final cell fate only based on the observation of the MAPK/ERK signalling pathway alone (136). Taking into account the physiological effects of all compounds mentioned, they may be considered bioactive in terms of their regulatory effects on cancer progression. In addition, in normal non-cancerous cells, most of these *Aloe* compounds did not have any effects, with the exception of the *A. arborescens* crude extract, aloin and aloesin.

MAPK/JNK/p38 pathway. Compared with the MAPK/ERK pathway, which is typically activated by mitogenic stimuli, the MAPK/JNK/p38 pathway is normally activated in response to stress signals and frequently observed to be deregulated in cancer. This signalling pathway is initiated following the activation of the respective receptors, followed by the triggering of megakaryoblastic leukaemia1/2/3-mitogen-activated protein kinase kinase (MEK)4/7-JNK1/2/3 or MEK1/4-MKK3/6-p38 cascades (132,136,137).

Similar trends as those observed in the MAPK/ERK signalling pathway were also observed in the MAPK-JNK/p38 signalling pathway regarding the effects of *Aloe* phytochemical compounds. The phosphorylation of p38 was reported to be increased, whilst p-JNK was not changed in HUVECs following aloe-emodin treatment combined with photodynamic therapy, while no effect was obtained with aloe-emodin treatment alone (138).

Emodin induced the phosphorylation of p38, whilst mildly suppressing the phosphorylation of JNK in SMMC-7721 cells (62). In another study, emodin inhibited the activation of p38 MAPK in the lungs of Wistar rats exposed to cecal ligation and puncture (139).

Of note, phosphorylation of p38 and JNK was decreased in BT-474 cells but increased in MCF-7 cells in response to chrysophanol (73).

Aloin was indicated to significantly increase the levels of phosphorylated c-Jun and p38 in A549 cells after 48 h of treatment (81). Aloin also reduced LPS-induced phosphorylation of p38 in RAW264.7 cells (85), but phosphorylation levels of p38 and JNK were not affected by aloin treatment in the same cell line in another previous study in the absence of LPS (83). Aloin suppressed D-gal-induced p38 phosphorylation in the hippocampus of healthy mice but did not affect the total levels of p38 (134). In addition, aloin suppressed PolyP-enhanced vascular permeability in HUVECs whilst inhibiting the PolyP-induced upregulation of p38 phosphorylation (106). Aloin also significantly inhibited TGFBIp-induced activation of p38 MAPK in HUVECs, but did not exert any effects on p38 in HUVECs without TGFBIp induction (107). The mRNA levels of cyclooxygenase-2 (COX-2), p38 and JNK were not significantly changed in kidney tissues of healthy mice after aloin treatment. However, cecal ligation and puncture surgery resulted in an increase in COX-2, p38 and JNK mRNA expression, whilst post-surgery treatment with aloin resulted in the reversal of this effect (140).

Aloesin decreased the phosphorylation levels of JNK and p38 in SKOV3 cells (87). However, aloesin treatment of the non-cancerous cell lines HaCaT and RAW264.7 did not change the levels of p38 in neither their phosphorylated nor non-phosphorylated forms, but increased JNK phosphorylation (135).

Isovitexin treatment inhibited LPS-stimulated phosphorylation of JNK1/2 and p38 in RAW264.7 cells, but did not have any effect on RAW264.7 cells without LPS stimulation (97).

TGF-β pathway. The TGF-β signalling pathway serves a pivotal role in cells and has been reported to be involved in controlling embryogenesis, immunity, angiogenesis and cell motility and to exert anti-proliferative and pro-apoptotic effects (141,142). However, an oncogenic role of this signalling pathway has been observed during the later stages of cancer development, exerting opposite functions in cancer cells compared with those in healthy cells (143,144). The TGF-β family of ligands normally bind to the constitutively active type II TGF-β receptor, which results in the recruitment of type I receptors and the formation of receptor complexes. Activated type I receptor phosphorylates receptor-regulated SMADs, which in turn form a complex with SMAD4. This complex translocates into the nucleus and activates the TGF-β-responsive element to initiate gene transcription. SMAD6 and SMAD7 serve as inhibitors of this signalling pathway (145).

In primary mouse embryonic fibroblast cells, it was indicated that *A. vera* inner gel upregulated the expression of TGF-β1 and basic fibroblast growth factor over the first 12 h of exposure (146). However, after 24 h, their expression was downregulated (146). Topical application of *A. vera* gel onto cutaneous wounds of rats increased TGF-β gene expression in the wound bed on days 4, 7 and 14 compared with that in the untreated controls, ultimately accelerating the wound-healing process (147).

Aloe-emodin attenuated unilateral ureteral obstruction-induced renal interstitial fibrosis *in vivo*. The upregulation of the protein levels of TGF-β1, collagen I, collagen IV and fibronectin in the renal tissues of mice following unilateral ureteral obstruction was significantly reversed after aloe-emodin treatment. The effects of aloe-emodin were comparable to those of the standard drug losartan. Application of aloe-emodin *in vitro* ameliorated renal interstitial fibrosis in TGF-β1-induced HK-2 cells by reducing the protein and mRNA expression levels of TGF-β1, collagen I, collagen IV and fibronectin to basal values (32). Emodin downregulated the expression of TGF-β receptor II in HeLa and SiHa cells. In addition, emodin downregulated SMAD4 and inhibited the phosphorylation of SMAD2 and SMAD3 in HeLa cells, without affecting the total SMAD2 and SMAD3 expression levels (61).

Aloesin treatment of a mouse wound excision model resulted in increased phosphorylation of both SMAD2 and SMAD3 in the wound tissue samples on day 7 post-injury without altering the expression of total SMAD2 and SMAD3 (135). Aloesin did not have any effect on the expression of SMAD7 (135). In addition, TGF-β1 expression was increased in RAW264.7 cells following treatment with aloesin (135).

A. vera glucomannans was indicated to normalise the decreased levels of TGF-β and IL-10 in the colon tissue in

a dextran sodium sulphate (DSS)-induced colitis model, to levels comparable with those in the control group. However, *A. vera* glucomannans did not have any effect on TGF-β levels in colon tissues of healthy animals, but promoted IL-10 production (148).

There is limited information available on the effect of *Aloe* phytochemical compounds on the TGF-β signalling pathway in cancer models (Table I and Fig. S3). Instead, more pronounced effects were demonstrated on its regulation of tissue regeneration.

Wnt and Notch pathways. Under physiological conditions, β-catenin is constitutively phosphorylated by glycogen synthase kinase 3β (GSK-3β) and casein kinase 1 (CK1) to be degraded by the complex consisting of axin, adenomatous polyposis coli (APC), GSK-3β and CK1. Upon activation of the Wnt cascade, β-catenin becomes stabilised and translocates into the nucleus to activate target gene transcription. The Wnt signalling pathway has been reported to regulate cell proliferation, survival and differentiation (149-151).

Peng *et al* (152) previously studied differentially expressed alternative splicing genes and their corresponding transcript variants in the human colorectal cancer cell line RKO after treatment with *A. vera* aqueous extract, isolated aloin and aloesin. Genes associated with activation of the Wnt pathway (co-activator, key factor and target genes) and inhibition of the Notch pathway (co-repressor genes) were indicated to be upregulated (152). However, the aqueous extract of *A. vera* and aloin were not able to directly activate the Wnt-signalling pathway in human colon cancer cell lines (153).

Aloe-emodin treatment was observed to inhibit EGF-induced phosphorylation of GSK-3β in a dose-dependent manner in a JB6 CI 41-5a cell neoplastic model (110).

Emodin downregulated the Wnt/β-catenin signalling pathway in HeLa cells by inhibiting β-catenin and GSK-3β (ser9) phosphorylation and reducing total GSK-3β protein levels (61). In addition, emodin was able to significantly inhibit the expression of β-catenin (CTNNB1) and transcription factor (TCF)-4, also known as TCF7 like 2, in SW480 and SW620 cells (71).

Esculetin significantly reduced the mRNA and protein levels of β-catenin in SMMC-7721 cells. The total β-catenin protein levels were not evidently decreased but the phosphorylated β-catenin levels (Ser33/Ser37/Thr41) were increased (113).

Aloesin upregulated the protein expression of β-catenin in Super TCF (STCF) NIH/3T3 cells, which were transfected with the STCF reporter gene plasmid and in human colon cancer HCT-15 and RKO cells. Aloesin was also demonstrated to upregulate the mRNA expression of cyclin D1 (CCND1), CTNNB1 and AXIN2 in Super TCF NIH/3T3, c-MYC, matrix metalloproteinase (MMP)-7, dickkopf WNT signalling pathway inhibitor 1, AXIN2 and APC in HCT-15 cells and c-MYC, CCND1, GSK-3β, AXIN2 and APC in RKO cells (153). These findings provided certain ambiguities on the possible carcinogenic effects of aloesin on the colon. However, no cell proliferation or cell cycle progression was observed after aloesin treatment in RKO cells. It is also noteworthy that the aforementioned gene expression data should be verified with protein expression and cascade activation assays.

A number of *Aloe* phytochemical compounds have demonstrated the ability to suppress the Wnt signalling pathway in cancer models (Fig. S3). However, certain discrepancies on the effects exerted by aloesin require further confirmation.

Suppression of the Notch pathway was also demonstrated for aloesin, aqueous extract of *A. vera* and aloin (153). After treatment with the aqueous extract of *A. vera* (12.5 μg/ml) and aloin (1 μM), the mRNA level of NOTCH1, NOTCH2 and hes family bHLH transcription factor (HES)5 in Super TCF NIH/3T3 cells were indicated to be significantly decreased. The aqueous extract of *A. vera* downregulated the mRNA expression of NOTCH1, jagged canonical Notch ligand 1 and HES1 in RKO cells and NOTCH1 and HES1 in HCT-15 cells. In addition, aloin downregulated the mRNA expression of NOTCH1 in RKO and HCT-15 cells, whilst aloesin downregulated the protein expression of NOTCH1 in RKO and HCT-15 cells (153).

6. Angiogenesis

Formation of new blood and lymph vessels is a major event during the development of all types of tissue, which is necessary for the delivery of oxygen, supply of nutrients and removal of metabolic waste products. In the case of tumorigenesis, increased angiogenesis is associated with the growth of the tumour and metastasis (154). A number of signalling pathways have been reported to be involved in angiogenesis stimulation, including PI3K/AKT, JAK/STAT and MAPK (155).

The number of newly-formed blood vessels was markedly reduced in the tumour tissue of L-1 sarcoma-bearing BALB/c mice after administration of the *A. vera* gel (156). By contrast, topical application of 1 and 2% *A. vera* cream activated angiogenesis, with its levels peaking on day 5, in a full-thickness wound model in Sprague Dawley rats (157). *A. vera* extract treatment of mouse fibroblast cells for 24, 48 and 72 h also slightly upregulated the expression of the VEGFA gene (158).

Matrigel® HUVEC tubule formation assays were previously performed to study the effect of aloe-emodin alone and aloe-emodin combined with photodynamic therapy on capillary blood vessel structure formation. Aloe-emodin combined with photodynamic therapy reduced the number of branching points and tubules in addition to reducing the tubular length, whilst aloe-emodin alone exerted no significant effects on vessel formation. In addition, aloe-emodin alone did not confer any effects on VEGF expression or ERK, whilst aloe-emodin combined with photodynamic therapy significantly reduced the level of these two parameters (138). In a BALB/c mouse burn wound model, application of 100 mg aloe-emodin ointment per mouse, at a concentration of 100 or 500 ng/ml, increased VEGF expression in the burn wound tissues on days 1 and 3 (159).

Emodin treatment downregulated the angiogenesis index of endothelial cells isolated from implanted pancreatic cancer tissues *in vitro,* whilst also suppressing angiogenesis in orthotopically implanted pancreatic cancer tumours, as detected by tumour vascular imaging. In addition, emodin treatment significantly decreased the expression levels of endothelial markers CD31 and CD105, but downregulated the protein expression of VEGF. However, no effect was observed on VEGF receptor (VEGFR) expression in the orthotopically implanted

pancreatic tumour tissues (104). In rats with jejunum injury, the JAK1/STAT3 signalling pathway in the jejunum tissue was markedly suppressed (160). However, after treatment with emodin, JAK1/STAT3 signalling activation was restored to levels comparable to those in the control group (160).

Chrysophanol significantly suppressed the expression of CD31, CD34 and angiogenin in tumour tissues from an A549 xenograft nude mouse model (73). Chrysophanol treatment of HCT116 and SW480 cells also led to a pronounced suppression of hypoxia-inducible factor 1α (HIF-1α) protein expression downstream of hypoxia induction (121). The expression of CD31 and the protein levels of HIF-1α and VEGF were reduced, whilst the protein level of vasohibin was increased by chrysophanol in a dose-dependent manner according to results from a HUVEC tubule-formation assay (72).

The expression of c-Myc and VEGF, which promote proliferation and angiogenesis, respectively, was significantly reduced in response to aloin treatment in SW620 cells. These *in vitro* results were confirmed in the SW620 xenograft nude mice model *in vivo*, where a tumour growth inhibition of 63% was observed on day 27, with reduced numbers of CD31-positive cells and increased numbers of apoptotic cells (82). The relative protein expression of VEGF was also significantly reduced in HepG2 and Bel-7402 cells following aloin exposure. Furthermore, immunohistochemical staining of orthotopic xenograft HepG2 tumours in nude mice revealed reduced VEGF expression in the tumour tissues *in vivo* (80). Aloin also inhibited HUVEC cell proliferation, migration and tubule formation *in vitro* by downregulating VEGF-induced VEGFR2 phosphorylation at the Tyr1175 residue (82). Treatment with 400 μM aloin did not alter the barrier integrity of HUVEC cell monolayers *in vitro,* but it suppressed the migration and adherence of neutrophils and inhibited leucocyte migration in a cecal ligation and puncture-induced septic mouse model *in vivo* (107).

In a previous study, aloesin was also observed to cause a dose-dependent stimulation of angiogenesis in the HUVEC tubule formation assay *in vitro* (135).

Acemannan upregulated VEGF expression in a dose-dependent manner in human deciduous dental pulp cells (161) and in primary human gingival fibroblasts (162).

Inhibitory effects of phytochemical compounds from *Aloe* on new vessel formation were observed. Although *Aloe* extracts have been indicated to demonstrate excellent wound healing properties, isolated phytochemical compounds do not appear to directly stimulate angiogenic mechanisms in normal cells, except for the *A. vera* extract and acemannan.

7. Migration and invasion

According to the TNM Classification of Malignant Tumours, late stages of cancer, namely stage III and IV, are defined as lymph node spreading or metastases (163), which reduces the probability of successful treatment. Cell migration and invasion are normal physiological processes that are important for development and regeneration (164). However, malignant cells use these processes for spreading across the body to form secondary tumours and to block or avoid 'stop'-signals (164).

CD82, also known as KAI-1, is a tetraspanin membrane protein that inhibits metastasis in human cancers (165,166). It was observed that CD82 mRNA expression was increased by ~4-fold in PC-3 cells after treatment with aqueous whole-leaf extract of *A. vera* (99).

Key mesenchymal markers such as Snail, Slug and N-cadherin were indicated to be downregulated following emodin treatment in HeLa cells (61). In addition, Lin *et al* (104) reported downregulation of MMP-2 and MMP-9 expression by emodin therapy in orthotopic pancreatic cancer tissues (104). Pre-treatment of four human glioma cell lines, U87MG, U373MG, U251MG and HS683, with 20 μM emodin abolished hyaluronic acid-induced cell invasion and migration *in vitro* without inducing cellular toxicity, whilst MMP-9 and constitutive MMP-2 secretion were suppressed in a concentration-dependent manner (120). Oral administration of emodin also effectively suppressed MMP-9 expression in mice bearing U87MG tumours *in vivo* (120). In addition, in W480 and SW620 cells, emodin treatment resulted in the suppression of the expression of mesenchymal markers Snail and vimentin, MMP-2 and MMP-9 enzymes, but increased the expression of E-cadherin (69).

Chrysophanol inhibited the migration and invasion of A549 and H738 cells in a dose-dependent manner. The protein levels of MMP-2, MMP-9, N-cadherin and vimentin were all indicated to be decreased, whilst those of tissue inhibitors of metalloproteinases (TIMP) and E-cadherin were markedly increased by chrysophanol in a dose-dependent manner in A549 and H738 cells and in HUVECs (72). In addition, chrysophanol was able to significantly attenuate hypoxia-induced cell migration and invasion in the human colon cancer cell lines HCT116 and SW480 *in vitro* by downregulation of MMP-2 and MMP-9 and upregulation of TIMP-3. Furthermore, chrysophanol prevented epithelial-to-mesenchymal transition by reversing the supressed E-cadherin expression and reversing the upregulation of N-cadherin and vimentin in hypoxia-induced cell migration and invasion models in HCT116 and SW480 cells. In an SW480 xenograft BALB/c nude mouse model, E-cadherin expression was indicated to be increased, whilst the expression of N-cadherin and vimentin was decreased in tumour tissues after chrysophanol treatment (121).

Aloin suppressed the migration and invasion of BGC-823 and HGC-27 cells after 24 h of treatment by reducing the protein expression levels of MMP-2, MMP-9 and N-cadherin, whilst simultaneously upregulating E-cadherin expression (78). Aloin also reduced the number of invasive HepG2 and Bel-7402 cells according to results from Transwell and wound-healing assays by reducing MMP-9 expression (80).

Aloesin treatment inhibited the migration and invasion of SKOV3 cells, in addition to downregulating MMP-2 and MMP-9 protein expression in a dose-dependent manner. In an experimental model of pulmonary metastasis in immunodeficient nude mice, the number of metastatic lung colonies was significantly reduced by aloesin treatment (87).

8. Inflammation and immunomodulation [TNF-α/nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) pathway]

TNF is a double-edged sword due to conflicting reports on the effects of this signalling pathway, including proliferation, angiogenesis, interactions with the immune system, survival

and apoptosis, which is dependent on the type of cancer in question (167). NF-κB is a heterodimeric complex that is usually comprised of the p50 and p65 subunits, which is associated with its cytoplasmic inhibitor of κB (IκB). After TNF-α binds to its receptor, TNF receptor (TNFR) superfamily member 1A associated death domain binds to the intracellular domain of the receptor and recruits TNFR-associated factor (TRAF)2, which is a type of receptor-interacting protein. This complex then recruits and activates IκB kinase (IKK), which phosphorylates IκB and causes it to dissociate from p65, preventing NF-κB from degradation. This released NF-κB then translocates into the nucleus to activate target gene transcription (168,169). However, other factors and receptors may activate the non-canonical TNF-α pathway without activating NF-κB but will instead lead to cell death (see section 2) (170,171).

Emodin downregulated the activation of NF-κB in a dose-dependent manner in SW1990 and PANC-1 cells (65,70,104). In flagellin-stimulated HT-29 cells, it was revealed that emodin downregulated the expression of toll-like receptor (TLR)5 and myeloid differentiation primary response 88 (MyD88), upregulated the expression of IκB, inhibited the nuclear translocation of NF-κB p65 and reduced the production of IL-8 (172). Pre-treatment of U87MG cells with emodin also significantly reduced NF-κB activation in a hyaluronic acid-induced cell invasion model *in vitro* (120).

Chrysophanol treatment resulted in the downregulation of IκB and p65 NF-κB phosphorylation in MCF-7 and MDA-MB-231 cells (71).

The phosphorylation and subsequent activation of IκBα and NF-κB was suppressed by aloin treatment in BGC-823 and HGC-27 cells (78). Aloin pre-treatment also ameliorated the damaging effects of dimethylhydrazine, a colon carcinogen, on Wistar rats through a protective mechanism that involved reducing the expression of oxidative stress enzymes glutathione peroxidase, glutathione-S-transferase, glutathione reductase and superoxide dismutase, reducing the expression of COX-2, inducible nitric oxide synthase (iNOS), IL-6 and proliferating cell nuclear antigen protein and inhibiting TNF-α release (173).

Esculetin treatment of PANC-1, MIAPaCa-2 and AsPC-1 cells significantly decreased the protein expression of NF-κB within 8-12 h without affecting that of its inhibitor IκB (88). After treating PC-3 cells with esculetin, the mRNA expression of TNFR1 was increased (89).

The described effects of the *Aloe* phytochemical compounds on the TNF-α pathway in cancer models are depicted in Fig. S3. However, *Aloe* phytochemical compounds also exerted immunomodulatory effects on normal cells in the absence of adverse effects in healthy animals.

Freeze-dried *A. vera* inner gel was indicated to significantly reverse *Shigella flexneri*- and LPS-induced TNF-α and IL-1β production to baseline levels in human peripheral blood leukocytes and THP-1 cells *in vitro* (174). The levels of TNF-α and IL-6 in the serum of Wistar Furth rats with second-degree burns were significantly decreased in the group treated topically with *A. vera* gel (175).

Treatment with aloe-emodin reduced the levels of inflammatory cytokine expression (IL-1β, IL-6, TNF-α, intercellular adhesion molecule 1 and vascular cell adhesion protein 1), TLR4 and NF-κB phosphorylation in a palmitic acid-induced inflammation model in the embryonic rat heart-derived cell line H9C2 (176).

Emodin treatment reduced the expression levels of TNF-α, IL-6 and monocyte chemoattractant protein 1 (MCP-1) in primary mouse fibroblasts from the hypertrophic scar tissue in C57BL/6 mice. By contrast, in normal primary mouse fibroblasts, no significant changes were observed in the expression levels of TNF-α, IL-6 or MCP-1 after emodin treatment (103). MCP-1 is secreted by numerous cell types, including monocytes, endothelial cells, smooth muscle cells and fibroblasts, as an initiator cytokine of the inflammatory cascade (177). Emodin was able to inhibit sepsis-induced secretion of pro-inflammatory cytokines IL-6 and TNF-α into the serum of rats with jejunum cecal ligation and puncture (160) and lung cecal ligation and puncture (139). The protein expression of TLR5 and NF-κB p65 was increased in the colon tissues of C57BL/6 mice following induced ulcerative colitis, which was in turn downregulated back to basal values after emodin treatment (172).

Aloin reduced PolyP- (106) and TGFBIp- (107) induced protein expression of TNF- α and IL-6, whilst downregulating the activation of NF-κB in HUVECs. In aloin-treated mice following cecal ligation and puncture, the levels of plasma NO, TNF-α, IL-6 and myeloperoxidase was normalised (140). Aloin significantly reversed cecal ligation and puncture induced iNOS protein expression, translocation and activation of NF-κB p65 in the nucleus and IκB phosphorylation in kidney tissues of mice. However, aloin did not exert any adverse effects on NF-κB signalling in healthy mice (140). In LPS-treated C57BL/6 mice, aloin significantly alleviated lung injury and reduced the levels of TNF-α and iNOS production in the broncho-alveolar lavage fluid, but intravenous aloin administration did not result in any adverse effects in intact animals (128). In another study that was performed on a similar LPS-treated mouse model, aloin A suppressed LPS-induced phosphorylation of NF-κB p65 and IκBα, whilst also suppressing the mRNA expression of TNF-α, IL-1β and IL-6 in the lung tissues of mice. However, aloin A did not exert any toxic effects on lung tissues of normal mice or alter p-NF-κB and p-IκBα levels in the lung tissue (84).

In LPS-stimulated RAW264.7 cells, the levels of pro-inflammatory cytokines and mediators, including TNF-α, IL-1β, IL-6, NO and iNOS, were inhibited by aloin, but no changes were observed in these mediators after aloin treatment without LPS stimulation (83-85).

Aloin was indicated to inhibit inflammatory responses in a hepatic ischemia-reperfusion model in C57BL/6 mice *in vivo* and in a hypoxia/re-oxygenation model in primary mouse hepatocytes *in vitro* by suppressing the NF-κB signalling pathway. The protein expression levels of MyD88, TRAF6, p-IKKα/β and p-NF-κB p65 in liver tissues or in primary hepatocytes returned to basal values after aloin treatment (86).

TNF-α, IL-1β and IL-6 levels in hippocampal tissues in the D-gal-induced mouse model of cognitive dysfunction were decreased after aloin treatment. Furthermore, aloin suppressed D-gal-induced NF-κB activation in the hippocampus (134). Aloin dose-dependently inhibited the expression of TRAF6, IKKα, IKKβ and NF-κB p65, whilst reducing the DNA-binding activity of NF-κB in an osteoclastogenesis model produced in

RAW 264.7 cells *in vitro* (178). Treatment with aloesin reduced TNF-α levels in RAW264.7 cells (135).

Treatment with isovitexin inhibited LPS/D-gal-induced TNF-α expression and NF-κB/IκB phosphorylation in the serum samples and liver tissues of mice (179). In LPS-stimulated RAW264.7 cells, treatment with isovitexin inhibited the production of cytokines TNF-α and IL-6, suppressed iNOS and COX-2 expression, blocked IκBα phosphorylation and reduced the nuclear levels of NF-κB p65. Isovitexin did not demonstrate any adverse effects on the aforementioned mediators in RAW264.7 cells in the absence of LPS stimulation (97).

A. vera glucomannans reversed the increased levels of cytokines IL-6, TNF-α and IFN-γ in colon tissues from a DSS-induced colitis mouse model group to levels similar to those in the control group. However, *A. vera* glucomannans did not mediate any effects on the cytokine levels in animals without this pathology (148).

Aloe polysaccharides reduced levels of IL-8 and IL-12 in TNF-α-induced HaCaT cell lysates and cultured media. Furthermore, *Aloe* polysaccharides attenuated TNF-α-induced expression of p65 NF-κB with simultaneous upregulation of IκBα expression in HaCaT cells (101). Im *et al* (180) previously demonstrated a dose-dependent increase in TNF-α production, IL-1β and NO release by RAW267.4 cells cultured with protein-free modified *Aloe* polysaccharides (fraction G2E1D).

Acemannan significantly upregulated IL-6 and IL-8 mRNA expression in human primary gingival fibroblasts and increased IL-6 and IL-8 protein secretion (181). Pugh *et al* (182) reported that pharmaceutical-grade acemannan prepared using a series of alcohol precipitation and centrifugation steps contained chemically similar components, such as aloeride, that exhibit immunostimulatory activities (182). Aloeride was indicated to induce the expression of IL-1β and TNF-α mRNA in the human monocytes cell line THP-1 *in vitro* (182).

Acemannan induced the production of NO in normal chicken spleen cells and the chicken bone marrow-derived macrophage cell line HD11, which was most likely mediated through mannose receptors (183). Acemannan exhibited mitogenic activity at a concentration of 100 mg/ml when it was added to the culture of mouse total spleen cells (184). Acemannan stimulated IL-6 and TNF-α production and NO release by RAW267.4 cells (185).

Modulation of TLR signalling has also been documented to be a mechanism of *Aloe* extracts or phytochemical compounds. The expression of TLR-2 and TLR-6 mRNA was significantly suppressed by *A. vera* glucomannans treatment in normal mice. By contrast, the mRNA expression of TLR-9 was upregulated (148). The upregulated mRNA expression levels of TLR-2, TLR-4, TLR-6 and TLR-9 in the colon tissue of mice with DSS-induced colitis were attenuated by *A. vera* glucomannans (148). In response to LPS, the level of TLR-4 was upregulated in RAW264.7 macrophages, which was reversed by aloin A treatment (84).

9. Recommendations for future applications

Pure *Aloe*-derived compounds have highly selective anticancer properties but do not appear to produce any severe adverse effects in non-cancerous cell lines. In addition, therapies combining established anticancer agents with *Aloe* compounds are a possibility that should be investigated in future studies. In such combination therapies, the dosage of the standard chemotherapeutic drug in question may be reduced, which in turn may reduce adverse effects. *Aloe* phytochemicals may enhance the anticancer effects of standard therapeutic drugs through synergistic effects or by other mechanisms, including increasing drug concentrations in the tumour tissues. In a previous study by Shen *et al* (57), a synergistic effect between aloe-emodin and gemcitabine in A549 cells was confirmed. Emodin monotherapy or combination with gemcitabine promoted cell apoptosis of the human pancreatic cancer cell line SW1990 and its derived gemcitabine-resistant cell line SW1990/Gem (65). In addition, it was indicated that either emodin treatment alone or emodin combined with gemcitabine in pancreatic tumour tissues from SW1990 cell-inoculated nude mice reduced the Bcl-2/BAX ratio, increased caspase-9 and caspase-3 activation and cytochrome *c* release, whilst reducing AKT (Ser473) levels (70). Isovitexin also inhibited cisplatin-induced renal inflammation by inhibiting TNF-α, IL-1β, IL-6 and malondialdehyde, ROS production and downregulation of NF-κB activation in kidney tissues (186).

10. Conclusions

The phytochemical components of different *Aloe* species have been documented for their healing effects in different diseases, particularly in cancer. Selected phytochemical compounds from different *Aloe* species have been reported to exert selective cytotoxic effects against cancer cells but not normal cells. The anticancer effects of some of these compounds have been demonstrated to be due to their influence on different signalling cascades and biochemical pathways. The limitation of the current review is that the mechanisms discussed are likely general downstream effects of *Aloe* compounds, despite most of the included studies having demonstrated their anticancer effect. Nevertheless, the data collected will be useful for further identification of direct targets. Hence, certain *Aloe* phytochemical compounds may be applied as highly efficient drugs to combat cancer, either alone or in combination with other anticancer drugs. In addition, plants from the *Aloe* genus remain to be a reservoir of bioactive compounds, which warrants further exploration.

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Availability of data and materials

The datasets used and/or anlayzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HS, JHH and CG made substantial contributions to manuscript conception and design, manuscript revision, critically

analyzed literature data. HS performed supplementary figure drawing. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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