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Title: Saponins in Cancer Treatment: Current Progress and Future Prospects

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Abstract

Traditional plants are known to contain a wide array of secondary metabolites with important biological activity, including anticancer activity. One of such metabolites is saponin; a steroidal or triterpenoid glycoside that is distinguished by its soap forming nature. Different saponins have been characterized and purified so far, and are gaining attention in cancer chemotherapy. Saponins possess incredible structural diversity which has been linked to their activity. They have been implicated in cancer chemoprevention and chemotherapy. Several studies have reported the role of saponins in cancer and their mechanism of actions including cell cycle arrest, antioxidant, cellular invasion inhibition, induction of apoptosis and autophagy. Despite the extensive research and significant anticancer effect of saponins there are no known FDA approved saponin based anticancer drugs due to a number of limitations including toxicities and drug likeness properties. Recent studies have explored options such as structural optimization, combination therapy and drug delivery systems to design saponins with increased efficacy and decreased toxicities. This review discussed the current knowledge on different saponins, their anticancer activity, mechanism of action as well as the current promising research on saponins within the last two decades and recommendations for future studies.

Keywords: Saponins; anticancer activities; traditional plants; mechanism of action; cell cycle arrest; apoptosis; chemopreventive; future cancer research

1.0 Introduction

Cancer is a group of diseases that is characterized by uncontrolled cell proliferation. This unconstrained cell growth has the potential to invade or spread to other parts of the body [1]. Genetic mutations can lead to cancer by accelerating cell proliferation and differentiation. Cancer is a global health challenge and is one of the leading cause of death in both developing and developed countries [2]. An epidemiological study conducted by the World Health Organization (WHO) noted that cancer accounts for the death of about 7.6 million individuals in 2018, and this figure was expected to double by 2030 [2]. Several therapies have been sought to treat cancer with chemotherapy being the most reported. This treatment involves using drugs/chemical agents to destroy rapidly dividing cells and ultimately prevent the spread to other normal cells of the body. Despite the success rate of chemotherapy, patients continue to suffer from several side effects (such as general weakness, fatigue, loss of appetite and infections). In addition, the lack of selectivity and toxicity of food and drug administration (FDA) approved anticancer drugs has resulted in a significant drawback in the treatment of cancer [3]. Therefore, the search for alternative therapeutic agents in the treatment of cancer is imperative.

Traditional plants contain phytochemical compounds, which are mainly secondary metabolites used by plants to ensure survival and fecundity. Phytochemical compounds of medicinal importance include glucosinolates, alkaloids, triterpenoid, flavonoids, saponins, pigments, and tannins. Various studies investigated the use of secondary plant metabolites in traditional medicine. These secondary metabolites displayed different biological activities such as antimicrobial, anti-inflammatory, cardioprotective, antiviral and anticancer properties. Approximately 60% of anticancer drugs in clinical use and pre-clinical trials (vinca alkaloids (vinblastine and vincristine), etoposide, paclitaxel, camptothecin, topotecan, irinotecan, curcumin, resveratrol, genistein, allicin, lycopene, diosgenin, beta-carotene, dactinomycin, bleomycin and doxorubicin, paclitaxel and camptothecin)

are derived from plants [4–6]. These plant-derived anticancer drugs are widely accepted and generally perceived as relatively safe in terms of toxicity.

Saponins are a structurally diverse class of phytochemicals naturally found in higher plants, marine organisms and microorganisms. This group has displayed various pharmacological properties, including anti-inflammatory, antiviral, cardioprotective, immunoregulatory effects, and anticancer activity [7, 8]. The profound impact of saponins on cancer has attracted significant interest in the pharmaceutical sector. These compounds have demonstrated outstanding potential in inhibiting different cancer cells under *in vitro* and *in vivo* conditions. Despite the substantial progress made in recent years, the use of saponins as an anticancer agent has faced certain drawbacks, mainly due to their toxicity and poor pharmacokinetic properties. Therefore, this review comprehensively evaluates the potential of saponin as an anticancer agent using various mechanisms; this includes the poorly studied pathways such as those involved in ferroptosis and necroptosis. Furthermore, the current knowledge on the use of saponins as a chemotherapeutic agent and the window of opportunities it presents for future research were also explored.

2.0 Classification of saponins

2.1 Based on sources

Saponins can be obtained from two primary sources, viz: natural and synthetic. Saponins acquired from natural organisms are termed “natural”, while those derived from the artificial route via laboratory synthesis are known as “synthetic”.

2.1.1 Synthetic saponins

Saponins are synthesized artificially by derivatization of saponins obtained from natural sources or via de-novo synthesis. Various natural saponins such as oleanane, ursane, lupane, dammarane, cholestane, spirostane, furostane and cardenolide can be synthesized chemically using numerous

techniques [9]. However, in certain instances, these procedures are marked by various limitations, including low yield, toxic chemicals and stringent reaction conditions. In recent years, the use of Schmidt trichloroacetimidate in activating sugars has, however, shown great potential [10]. Although the mechanisms involving the chemical synthesis of saponins are beyond the scope of this review, it should be noted that the synthetic approach associated with saponin purification from a natural source forestalls the challenge of low yield and purity [11]. Additionally, this methodology allows a structure-based optimization that will enable the design of saponins equipped with desirable structural features.

2.1.2 Natural Sources of saponins

Historically, saponins were primarily derived from vegetables and herbs. Herbs containing saponins include; soapwort, ginseng, ginsenosides, gypenosides, soapberry rhizomes, *Liliaceae*, *Dioscoreaceae*, *Agavaceae*, *Primulaceae*, *Sapotaceae* and *Caryophyllaceae* [12, 13]. Furthermore, multiple types of saponin can be isolated within the same plant species. Saponins, although initially thought to be endemic to plants, can be found in non-plant sources. In the last three decades, marine organisms have been identified as significant sources of saponins. More specifically, organisms belonging to the phylum Echinodermata are rich sources of saponins. Tian et al. identified three groups of saponins (asterosaponins, cyclic glycosides and polyhydroxysteroidal glycosides) found in starfish and sea cucumbers [14].

2.2 Classification based on the structure

Saponins can also be grouped based on their structures. A typical saponin molecule is made up of distinct structural component consisting of an isoprenoid unit and a sugar residue. The former is referred to as the aglycone component, while the latter is called glycone. Acid hydrolysis of the glycosidic bond between glycone and aglycone of saponins can be used to separate these structural units. The unique structure of saponins and their amphiphilic nature is responsible for the biological

activities. It consists of a hydrophilic sugar moiety and a hydrophobic genin (called sapogenin). Additionally, aglycones may possess a structure of steroids or triterpenes, which are used to classify saponins.

Triterpenoid saponins (basic) consists of four or five rings, with a 30-carbon backbone structure derived from 2,3-oxidosqualene [15]. The pentacyclic triterpenoids are the most abundant triterpenoids in plants, and they include oleananes, lupanes, ursanes and their derivatives (such saikosaponins) (Figure 1). The less common tetracyclic triterpenoid saponins are dammaranes and their derivatives (including ginsenosides). On the other hand, the steroidal sapogenins are 27-carbon sugar conjugates of steroids consisting of a five- or six-ring skeleton known as spirostane and furostane, respectively. They include dioscin, diosgenin, polyphyllin D, timosaponin AII, cardenolide and cholestane (Figure 2).

Saponins also differ in structural composition, linkage and the number of sugar chains. Usually, the sugar chain may consist of one or more monosaccharide residues attached at C-3 [16]. Based on the number of sugar residues, saponins are classified as monodesmodic, bidesmodic and polydesmodic, if they contain one, two and more than two sugar residue, respectively. Saponins are also named based on the nature of the sugar residue present on their chain. Glucose containing saponins are regarded as glucosides, while galactose containing saponins are galactosides.

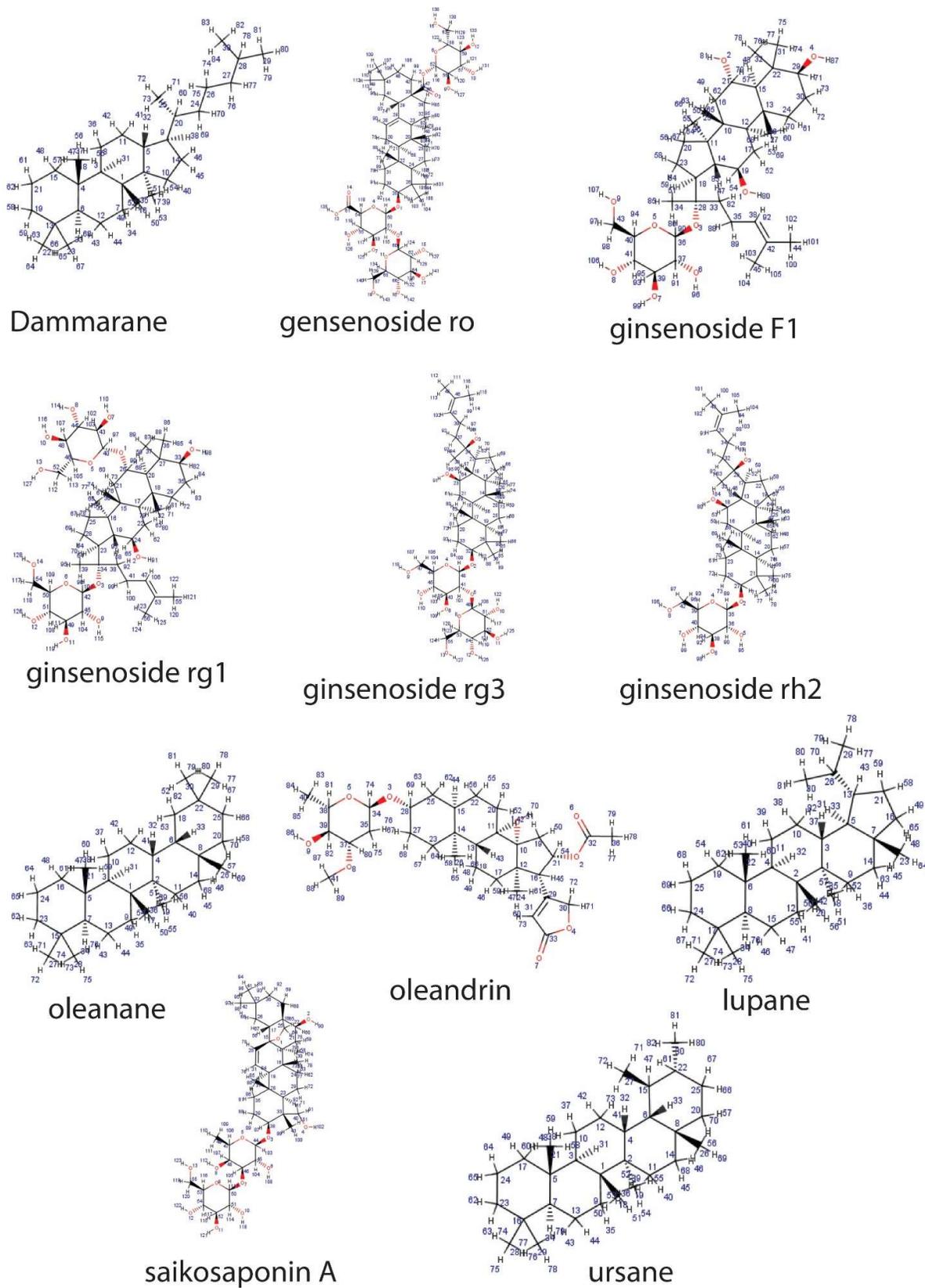


Figure 1: Representative sapogenin structure of triterpenoid saponins

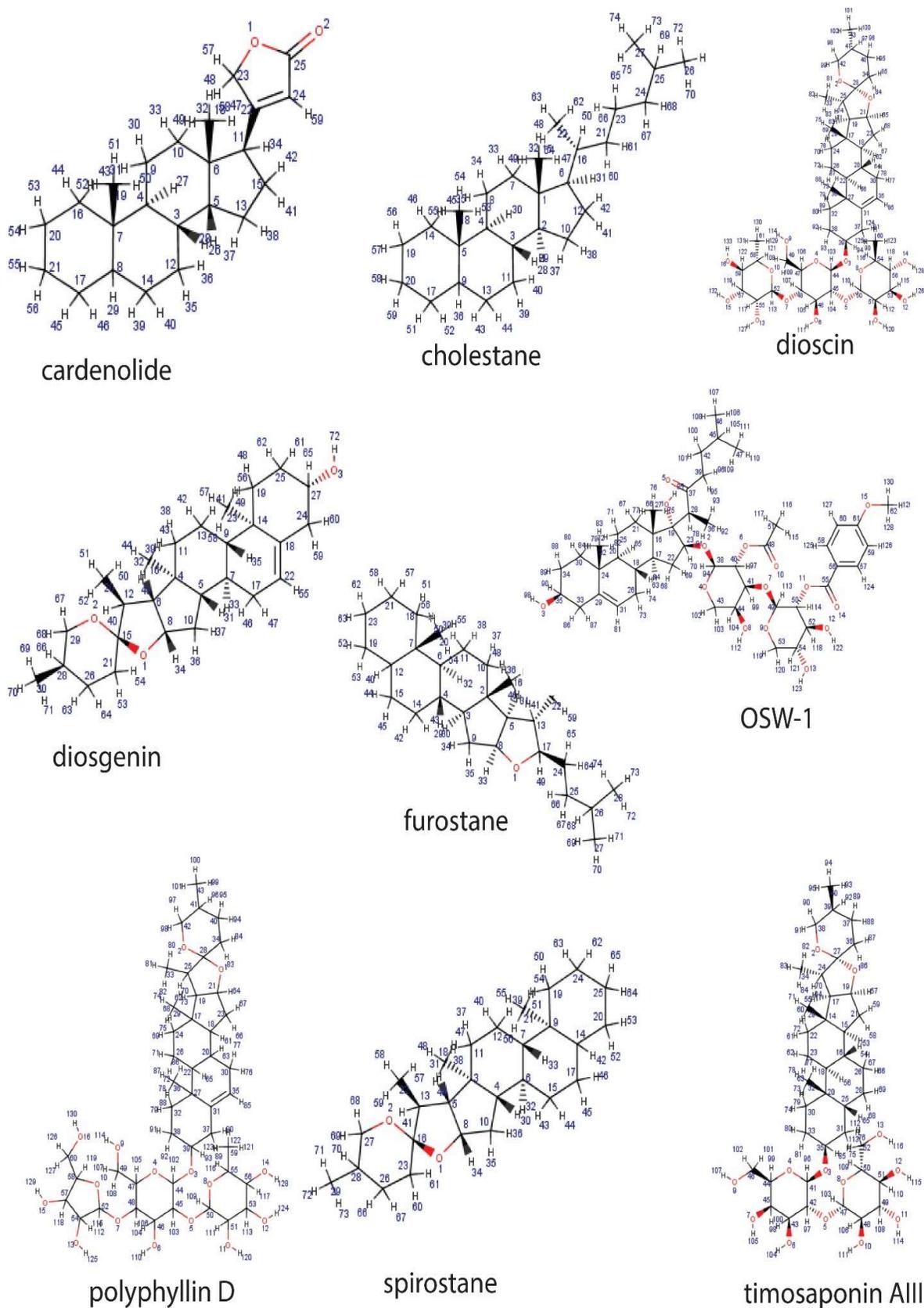


Figure 2: Representative sapogenin structure of steroid saponins.

3.0 Anticancer mechanisms of saponin

Existing research articles have shown that these unique class of phytochemicals derived from various plants exhibit anticancer activity [17]. Saponin prevents the proliferation of cancer cells by interfering with the replication of cellular DNA, thus reducing the risk of cancer in humans. The anticancer activities of saponin include anti-proliferation, anti-metastasis, anti-angiogenesis and reversal of multidrug resistance (MDR). They exert these effects through induction of apoptosis, promotion of cell differentiation, immune-modulatory effects, bile acid-binding and amelioration of carcinogen-induced cell proliferation [18]. Different molecular mechanisms are involved in the anticancer activity of saponins (**Table 1**). It should be noted that the mechanism of anticancer action of saponins is strongly related to the nature of the structural moieties including the aglycone moiety, the length and linkage of the glycosidic chain, the presence of a functional carboxylic group on the aglycone chain, the number of sugar molecules and hydroxyl group, position of the hydroxyl group, stereo-selectivity and the type of sugar molecule on the glycine chain [19–21]. Here, we consider the critical processes in cancer cell development and how different saponins help to inhibit cancer at various stages.

Table 1: Anticancer activities of Saponins and sapogenins

Compound	Cells/tissue type	Molecular target	References
Diosgenin	MCF-7, Breast cancer	The activation of p53, disruption of intracellular Ca ²⁺ homeostasis, generation of ROS and caspase activation	[22, 23]

Dioscin	Leukaemia, Lung cancer, Gastric carcinoma, Hepatocellular carcinoma, Cervical cancer, Breast cancer	Up-regulates FADD, p53, Bid and Bax. Downregulates CDK2, Bcl-2, Clap-1 and Mcl-1	[24–26]
Polyphyllin D	Ovarian cancer, Cervical cancer, Breast cancer, Glioblastoma, Glioma	Up-regulates p53, p21, PDI and JNX. Downregulates CDK1, Bcl-2, HIF- and VEGF	[27–30]
Oleandrin	Pancreatic cancer, Prostate cancer, Breast cancer, Lymphoma, Melanoma, Osteosarcoma	Upregulates Akt, ERK and ROS. Downregulates NF- κ B, MAPK, JNK, pS6, p4EPB1, PI3K/Akt and mTOR.	[31]
Ginsenoside Rg3	lung cancer, oesophagal carcinoma, gastric cancer, colon cancer, hepatoma, renal cancer, bladder cancer, breast cancer, ovarian cancer, prostate cancer and melanoma	Up-regulates p63,p21, Bax and Smac Downregulates VEGF, p38 and P13K,	[18]
Ginsenoside Rh2	Leukaemia, Colon cancer, Hepatocellular carcinoma,	Up-regulates p53, p21, p27 and p16	[18]

	Breast cancer, Ovarian cancer, Prostate cancer	Downregulates AKT, CDK4, CDK6 and AP-1.	
SaikosaponinA	Hepatocellular carcinoma, Breast cancer, Colon cancer	Up-regulates p15, p16, ERK and cleaved-PARP Downregulates Bcl-2, XIAP, Clap2 and Pgp	[32]
Saikosaponin D	Lung cancer, Hepatocellular carcinoma, Prostate cancer, Thyroid cancer	Up-regulates p53, p21, Fas and Bax, Downregulates Bcl-2, CDK2, COX-2 and STAT3	[33]
Polyphyllin D	Human non-small cell lung cancer NCI-H460 cell line.	ER stress-mediated apoptosis, induction of tumour suppressor p53, disruption of mitochondrial membrane and activation of caspase-9 and caspase-3	[34]
Timosaponin AIII (TAIII)	Breast, prostate, HepG2, pancreatic and osteosarcoma cancer cells. PANC-1 cell xenograft nude mice model	ER stress induction, activation of caspase-3, downregulation of Bcl-2, X-linked inhibitor of apoptosis protein (XIAP), Mcl-1 and IAPs, induction of cytochrome	[35–37]

		c and stimulation of caspase 3, 7, 8, and 9	
OSW-1(3 β ,16 β ,17 α -trihydroxycholest-5-en-22-one16-O-(2-O-4-methoxybenzoyl- β -D-xylopyranosyl)-(1 \rightarrow 3)-(2-O-acetyl- α -L-arabinopyranoside).	Leukaemia cancer and pancreatic cancer cell	Mitochondria membrane permeabilization. Intrinsic apoptosis. Calcium-dependent GRP78 (survival factor) cleavage. Binding to oxysterol binding protein to activate the Golgi stress response leading to apoptosis	[38–40]

3.1 Chemoprevention and saponin

Chemoprevention is the use of a chemotherapeutic agent to halt or restrict tumour development before the onset of cellular invasion. The chemopreventive action of saponins involves anti-inflammation, redox potential modulation and cell proliferation inhibition (**Figure 3**).

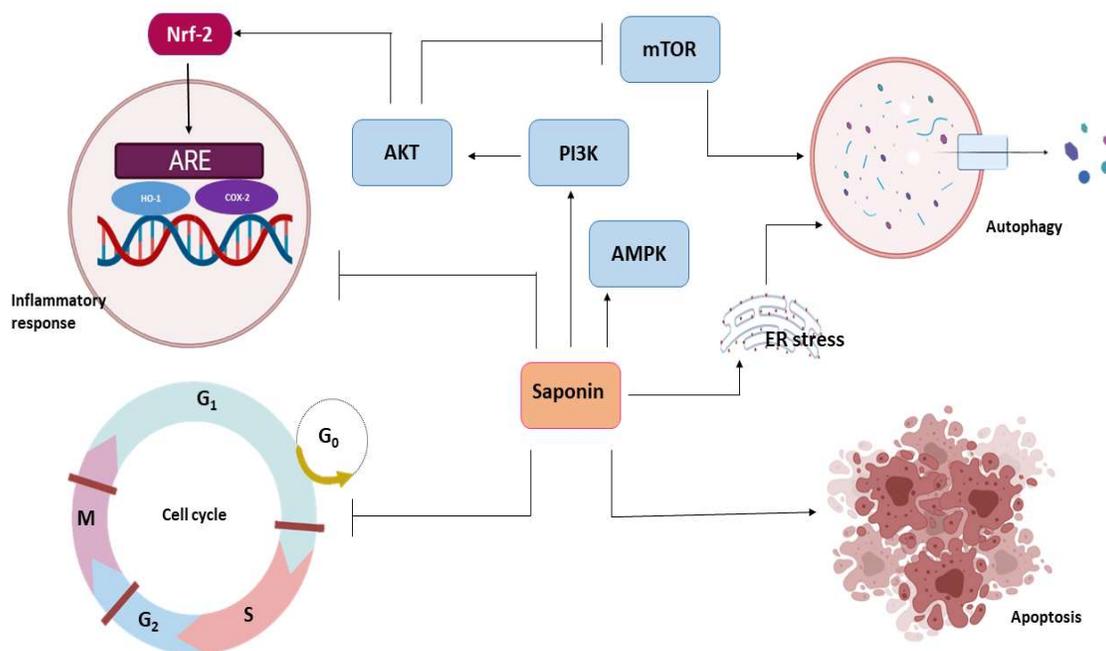


Figure 3: Anticancer effects of saponins

3.1.1 Anti-inflammatory activity

The body auto-immune system triggers inflammation in response to foreign invaders as part of the body defence mechanism. Nonetheless, excessive or chronic inflammation is associated with different pathological conditions, one of which is cancer [41]. Due to the link between cancer and inflammation, several anti-inflammatory drugs help to decrease the incidence of cancer. Most inflammatory drugs have been designed to selectively target vital proteins like nuclear factor Kappa B (NF- κ B), IL-6/STAT3, IL-23/Th-17 and cyclooxygenase -2 (Cox-2), which are responsible for inflammatory response. Like other anti-inflammatory drugs, some saponins are capable of regulating the expression of a number of these proteins.

NF- κ B is an inducible transcription factor that stimulates the expression of pro-inflammatory and pro-survival genes. They can be activated via a canonical pathway involving TNF- α , T-cell and B-

cell receptors. Triggering of this protein in cancer cell leads to activation of cell cycle proteins, metalloproteinase and apoptotic proteins. Several reports have identified saponins that inhibit NF- κ B and other proteins that function upstream of NF- κ B activation. For instance, Paris saponin II, a steroidal saponin, inhibits IKK-b, a protein involved in the canonical pathway of NF- κ B activation, leading to cell cycle arrest and apoptosis activation [42]. Also, Raddeanin A, a triterpenoid, inactivates NF- κ B by preventing the phosphorylation of I κ kb- α . A study by Xia et al. likewise reported the downregulation of the NF- κ B signalling pathway by saponins of *Patrinia villosa*, which led to a significant inhibition in colorectal cell proliferation, invasion and metastasis [43].

Inhibition of TNF- α and Cox-2 has been observed by saponin fractions from marine spiny brittle starfish extract [44]. Triterpenoid saponin from *Conyza blinii* showed tremendous anticancer activity via p65 dependent NF- κ B inhibition [45]. Bioactivity-guided isolation of compounds from *Cyclocarya paliurus* led to the isolation and identification of dammarane triterpenoid, which mediates anti-inflammatory activity by reducing the expression of TNF- α , PGE2, and IL-6 [46]. Structure dependent activity studies of different triterpenoid isoforms revealed cyclocarioside X as a potent chemopreventive agent by showing significant inhibition of COX-2, iNOS (inducible nitric oxide synthase), and NF- κ B/p65 in Raw 264.7 cells. The role of saponins in regulating proteins involved in inflammatory pathways undermines its critical chemopreventive potentials.

3.1.2 Modulation of redox potential

Reactive oxygen species (ROS) consists of free radicals involved in tumour cell proliferation, genomic instability, resistance to apoptosis and tumour invasion [47]. An imbalance between free radical production and the antioxidant defence system leads to oxidative stress implicated in cancer initiation. The redox imbalance can be reversed using saponins by acting as free radical scavengers, modulating the redox signalling pathway and increasing the expression of antioxidant enzymes. Saponins have been identified as potent free radical scavengers and can increase the expression of

antioxidant enzymes [48]. Purified bacosides, a triterpenoid saponin from *Bacopa monnieri*, has shown significant DPPH radical scavenging activity. Moreover, Choudhry et al. reported that saponin based nano-emulsification improves the antioxidant properties of Vitamin A and E in AML-12 cells [49]. Furthermore, *Panax notoginseng* saponins increase the expression of the antioxidant enzyme heme oxygenase-1 by increasing the phosphorylation of AKT protein and the activity of Nrf2 [50].

Saponins have also shown pro-oxidant activity in cancer cells, in addition to their antioxidative activity. Dysregulation of redox signalling is a feature in most cancer cells and cancer cells survive oxidative insult by upregulating antioxidant defence system via the antioxidant response element (ARE). Blocking cancer cell antioxidant defence systems would increase ROS-induced oxidative damage, resulting in cancer cell death [51]. Triterpenoid saponin from *Ardisia gigantifolia* causes cell death in triple-negative breast cancer by increasing the generation of reactive oxygen species, activating ERK and AKT and inducing apoptosis via the intrinsic pathway [52]. Kim et al. also observed that hederagenin obtained from ivy leaves mediates cell damage in the head and neck cancer cell by reducing glutathione reductase activity, increasing ROS and inhibiting the Nrf2-ARE pathway [53].

3.1.3 Cell cycle arrest

Cell progression through the cell cycle is mediated by crucial proteins such as cyclins, cyclin-dependent kinases (CDK) and regulated by checkpoint kinases such as Polo-like kinase, aurora kinase and CDK inhibitors [54]. Cancer cells often show mutations in protein kinases (CDK2, CDK4, CDK6, chk1, Wee1 and PLK1) involved in cell proliferation. Targeting these proteins has become an attractive chemopreventive strategy to mitigate abnormal cell proliferation in cancer cells [55]. Saponins have in recent years shown attractive anticancer potentials by modulating cell

cycle proteins including cyclins, cyclin-dependent kinases and the checkpoint proteins to terminate cancer cell progression.

Sequel to proliferative stimulus, cells in the resting stage (G₀) progresses through the G₁, S, G₂ and M phase of the cell cycle. Different saponins regulate cell progression at each phase of the cell cycle. Furostan-type steroidal genin from edible spears of triguero HT asparagus decreased the expression of cyclin A, D and E by mediating G₀/G₁ arrest in human colon cancer cells [56]. Similar cell cycle suppression at the G₀/G₁ phase has also been observed in Paris saponin VII treated human leukaemia cells (K562/ADR) [57]. The saponin decreased cyclin B1/D1 and CDK2/4/6 protein expression. Chikusetsu saponin IV, a methyl ester, a ginsenoside purified from *Panax japonica*, has similarly shown the capacity to decrease cell cycle progression through the –S-phase [58]. Moreover, the compound was shown to inhibit the expression of cyclin d1, CDK2, and CDK6. Yaoming et al. reported cell cycle arrest in the s-phase by triterpenoid saponin from *Camellia sinensis* in the human ovarian cancer cell [59]. The cellular inhibition was achieved by downregulating Cdc25A, Cdk2, and CyclinD1 expression. More so, Paris saponin I have shown G₂/M₁ arrest in gastric cancer cells by upregulating the activity of p21, a checkpoint protein [60]. Recently, a steroidal saponin purified from the rhizome of *Paris polyphylla* var. *latifolia* was shown to induce the expression of p21 and downregulated the expression of cdc25C, Cyclin B1 and cdc2, thereby inducing G₂/M phase arrest in human colorectal cancer [61].

In a normal cell, damage to cellular components (such as DNA damage) would stimulate cell cycle inhibition, thus preventing the progress of the cell through the cell cycle. However, cancer cells are unresponsive to proteins associated with the regulation of the cell cycle. Targeting checkpoint proteins such as ChK (checkpoint protein), p21 and Wee1 have become an attractive therapeutic target by many anticancer drugs [54]. Hellebrigenin, a steroidal saponin, induces G₂/M phase arrest by breakage of DNA strand and subsequent activation of ATM, Chk1 and Chk2, thus inhibiting downstream CDK1/Cyclin B1 kinase in HepG2 cells [62]. Diosgenin has similarly shown

modulation of the Cdc25C-Cdc2-cyclin B pathway by mediating G2/M cell cycle arrest in breast cancer [63].

3.2 Cytotoxicity effects of saponins

Saponins are a group of compounds that elicit cytotoxic action such as stimulation of autophagic cell death, decrease in nitric oxide production in cells and cytoskeleton integrity disassembly. Their cytotoxic effects can be initiated either by apoptosis or non-apoptotic stimulation of cell death. Extensive literature search has revealed the significant ability of saponins to induce cancer cell death through apoptosis, ferroptosis, oncotic necrosis, necroptosis and autophagy.

3.2.1 Apoptosis

Apoptosis is a programmed cell death characterized by cell shrinkage, chromatin condensation, nuclear fragmentation and membrane blebbing. It may be initiated either at the plasma membrane (extrinsic pathway) or inside the cell and critical in regulating tissue development and homeostasis [75]. Apoptosis is the most studied form of cell death and unlike other forms of cell death, it is well regulated and not accompanied by an inflammatory response. Induction of apoptosis of tumour cells is an effective way of treating tumours. Compelling evidence has shown that most cytotoxic agents used in cancer therapy can induce apoptosis [64].

Saponins can induce apoptosis through a series of reactions involving the activation of a protease family of enzymes known as caspase. Other caspases independent apoptosis pathways have also been described in the mechanism of cell death by saponins. In this section, we consider the cellular mechanism of cell death by saponins and elucidate the underlying molecular mechanism of the induction (**Figure 4**).

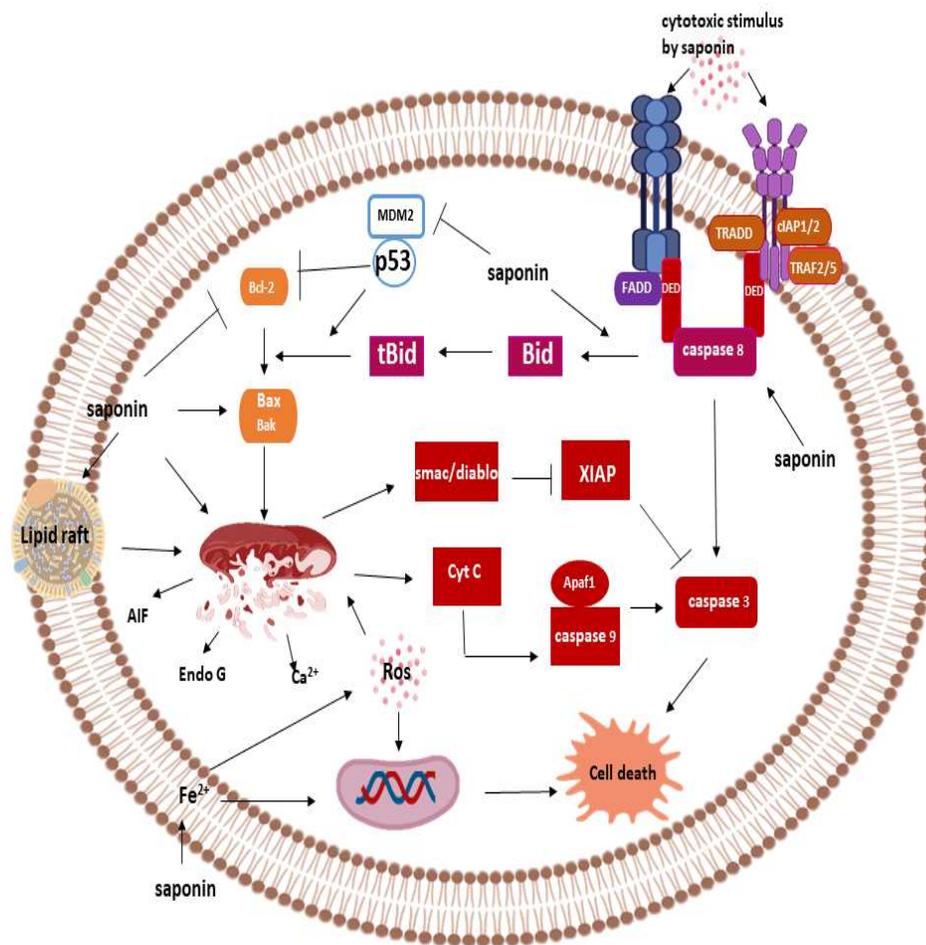


Figure 4: cell death mechanisms of saponins

3.2.1.1 Saponins and caspase-dependent apoptosis

Caspases are cysteine dependent aspartate specific proteases that mediate the initiation and execution phase of apoptosis. These enzymes are synthesized in their inactive form known as pro-enzyme or zymogens, and they can be activated via a receptor-mediated pathway or the mitochondria-dependent pathway. While the former is known as the extrinsic pathway, the latter also called the intrinsic pathway. Saponins can initiate a caspase-dependent pathway of apoptosis via the extrinsic and intrinsic pathway.

3.2.1.2 Extrinsic pathway and saponins

The extrinsic pathway is initiated by binding of ligand to members of the TNF superfamily of protein, including Fas receptor, TNF- α , and TRAIL. Saponins can activate the extrinsic pathway of apoptosis by activating the Fas receptor leading to the recruitment of adaptor molecule called Fas-associated death domain (FADD) [65]. The recruitment of FADD triggers the conscription of Procaspase 8 in saponin treated cancer cells to form death induced signalling complex (DISC) [64, 66]. Upon recruitment, procaspase-8 is released from DISC as the active caspase-8 via proximity induced activation mechanism. Activation of caspase-8 leads to downstream activation of the executioner caspase-3, poly-ADP-ribose polymerase (PARP) cleavage mediating the proteolysis of cellular component [67]. Caspase-8 activation is the defining factor in the extrinsic apoptosis pathway, and activation of this protein can be induced by different saponins [68].

Cellular activation of caspase-8 by saponins via the intrinsic pathway might not be sufficient to induce apoptosis [65]; as a result, some saponins rely on cell death machinery via the BCl-2 family of protein (Bid) which mediates crosstalk with the intrinsic apoptosis pathway [69]. Caspase-8 activate tBid by protein cleavage to form active Bid, which subsequently activates downstream pro-apoptotic proteins, Bax and Bak, causing mitochondria membrane permeabilization and activation of effector caspases [70]. Furthermore, Since p53 mutation in cancer cells can inhibit apoptosis in the intrinsic pathway, this pathway of cell death offers an alternative route of eliminating cancer in p53 mutant cells [70].

3.2.1.3 Intrinsic pathway and saponins

The intrinsic pathway is a mitochondria-dependent pathway of apoptosis, and it is the most reported mechanism of apoptosis induction by chemotherapeutic agents. Saponins can stimulate the release of pro-apoptotic factors, cytochrome C, Ca²⁺ and Smac/DIABLO, from the mitochondria via cytotoxic action or ROS production [71, 72]. These ROS/cytotoxic stimuli disrupt the mitochondria to initiate the apoptosis process. Pro-apoptotic cytochrome C binds Apaf-1 to form the apoptosome

complex required for the activation of pro-caspase-9. Upon activation, caspase-9 cleaves executioner caspase-3, activating the protein and the downstream apoptosis process. Activation of apoptosis by saponins via the intrinsic route involves the inhibition of anti-apoptotic protein Bcl-2 and activation of pro-apoptotic proteins caspase-9 and caspase-3 [73].

Saponins also mediate intrinsic pathway via mechanisms involving the activation of p53 proteins [74]. Activation of p53 can be achieved by the inhibition of MDM2 via direct interaction or by binding to the alternative reading frame (ARF) [75]. Activation of p53 causes the inhibition of anti-apoptotic Bcl-2 and activation of pro-apoptotic Bax, Noxa, and Bad leading to depolarization of mitochondria and the release of cytochrome C from the mitochondria [65]. This protein then mediates executioner caspase -3 and -9 activation [66]. Furthermore, saponin can stimulate Smac/Diablo to subsequently inhibits the activity of XIAP (inhibitor of executioner caspase-3), thereby stimulating apoptosis [68].

3.2.1.4 Saponin and Caspase independent apoptosis

Saponins are capable of inducing cell death via pathways independent of caspases but show morphology features typical of apoptotic cell death. In this form of cell death, caspases are not activated and their stimulation may not play any active roles in mediating cell death [76]. Sequel to the permeabilization of the mitochondria during mitochondria cell death pathway, different pro-apoptotic factors are released into the inter-membrane space, some of which are cytochrome C, Ca^{2+} , Smac/DIABLO, HtrA2/Omi, AIF (Apoptosis-inducing factor) and Endonuclease G. While some of the proteins mediate apoptosis via the intrinsic pathway as earlier discussed, AIF, HtrA2/Omi and Endo G translocate into the nucleus where they bind to DNA resulting in chromatin condensation.

Preceding the release of pro-apoptotic proteins (such as AIF) is the permeabilization of the membrane -this process plays a critical role in the overall apoptosis process and is termed as the

committed steps. One of the alternative pathway of cell death induction by saponins involve pore formation on the membrane [77]. Saponins are capable of binding to the cholesterol-rich segment of the membrane or the membrane lipid raft. The cytotoxicity of some saponins can be greatly influenced by the cholesterol content [78]. The binding of saponins to lipid raft might be the initial upstream process of mediating cytotoxic activity in multidrug-resistant cancer cells before the downstream release of pro-apoptotic Endo-G and AIF [79].

AIF and Endo G have become attractive targets due to their role in caspase-independent apoptosis. Saponins, including Dioscin, can induce caspase-independent apoptosis by activating AIF [26]. Although the activation of AIF by saponins is linked to increased ROS generation, a ROS independent mechanism activation has also been described [26, 80–82]. Also, saponins can stimulate the release of Endo G, resulting in their migration to the nucleus, where they bind to chromatin and break the phosphodiester linkage in the nucleotide chain to generate nucleosomal fragment [83, 84]. In addition to the pivotal role played by Endo G and AIF, HtrA2/Omi also mediates caspase-independent apoptosis. This pathway of death mechanism may prove an invaluable tool to destroy cancer cells resistant to caspase activation.

3.2.2 Ferroptosis, oncotic necrosis and necroptosis

Ferroptosis is an iron-dependent programmed cell death characterized by the accumulation of lipid peroxides [85]. Different saponins such as ardisiacrispin B, spirostanol saponin, diosgenin saponin, Oleanane triterpenoid saponin derivatives and ruscogenin have demonstrated iron-dependent programmed cell death following treatment on cancer cells [86–88]. Cancer cell depends on iron for DNA synthesis - an essential step in the cell cycle. Iron overload, however, can cause oxidative damage in cancer cells via the Fenton reaction [85]. This mechanism holds great promise to combat both drug-sensitive and resistant cancer cells [88].

Furthermore, Gao et al. discovered a novel form of cell death in tumour cells in which exposure to trisaccharide saponin derivatives induces cell swelling followed by cell membrane perturbation and destruction of the cytoskeletal network in the form of cell death known as oncotic necrosis [89]. Polyphyllin D and progenin III can induce programmed necrosis/necroptosis in cancer cells [27, 90, 91]. The molecular mechanism by which saponins exert necroptosis is not fully known, but like apoptotic cell death, it involves the activation of Caspase 8 as observed in the extrinsic pathway of apoptosis [27].

3.2.3 Autophagy and saponin

Autophagy is a mechanism adopted by cells to remove dysfunctional or redundant cellular components, which are later recycled to meet the metabolic needs of starving cells. It plays a dichotomous role in cancer cell death and pro-survival mechanism [92]. Autophagy induces cell death via apoptosis while they help cancer cells survive oxidative insults and metabolic stress by recycling dysfunctional cell component. Despite the significant progress made to understand the mechanism of autophagy, the question of whether to stimulate or inhibit autophagy in cancer therapeutics remains debatable [93]. Several studies on cancer have shown that autophagy promotes cell survival in cancer cells; however, excessive autophagy exceeding cellular repair capacity stimulates cell death [94]. While most anticancer agents seem to inhibit autophagy, some have also shown an ability to stimulate autophagy. Purified pulsatilla saponin D (SB365) from *Pulsatilla chinensis* showed a dual role by inducing the early event of autophagy (autophagosome formation) and inhibiting the latter stage of autophagy (autophagic flux) [95]. Zhang et al. noted that SB365 increased microtubule-associated protein 1A/1B-light chain 3 (LC3) expressions in HeLa cells. LC3 is involved in the formation of autophagosome; p62 can degrade them to inhibit autophagic flux. The authors, however, concluded that the inhibition of autophagic flux by increasing p62 expression might play a significant role in the anticancer activity of SB365 against HeLa cell.

Different molecular pathways, including mTOR, MAPK, AMPK and JNK, are implicated in the regulation of autophagy [96]. However, PI3/Akt/mTOR signalling pathway, which mediates crosstalk between autophagy and apoptosis, appears to be the most studied [94, 97]. Xie *et al.* reported the induction of autophagy by Paris saponins from *Paris polyphyllae* through the downregulation of Akt/mTOR in breast cancer cells [98]. Triterpenoid glycosides are also reported to induce apoptosis in hepatocellular carcinoma by modulating PI3K/Akt/mTOR signalling pathway [99]. Promoting autophagy through mTOR inhibition might be an effective way of cancer chemoprevention via management of metabolic stress [94, 100].

Autophagy is also involved in cell starvation via P13/AKT independent pathway [101]. Moreover, a mechanism involving ROS dependent autophagy through the treatment of cancer cells with saponins has been demonstrated [102]. Oxidative stress induces apoptosis in cancer cells via the MEK/ERK signalling pathway [103]. The saponins extracted from tea flower induced ROS dependent autophagy in ovarian cancer cells resulting in the activation of the MAPK signalling pathway [104]. Recent evidence has suggested that a specific protein known as AMPK can act downstream of MAPK to induce autophagy [105]. AMPK is an energy stress response protein that facilitates metabolic activity in cells to generate more ATP, which in turn causes oxidative stress through the generation of ROS. In NSCLC cells, treatment with Paris saponin VII was shown to increase the expression of AMPK and its downstream effector, ulk1, which are critical in inducing autophagy [106]. *In vitro* and *in vivo* studies have also demonstrated the induction of autophagy by saponins via the JNK pathway [107, 108].

Due to autophagic flux often associated with the growth of tumours, recent studies have primarily focused on identifying autophagy inhibitors [92, 93, 109]. A study by Liu *et al.* identified triterpenoid saponin from *Conyza blini* capable of eliciting cytotoxic activity in HeLa cells through the inhibition of autophagy [110]. However, the inhibition of autophagy may enhance the

recruitment of anti-tumour immunity [93]. Therefore, further studies are still needed to understand how saponins modulate autophagy to prevent cancer progression.

3.3 Metastasis and saponin

In some cases, tumour cells migrate from their primary site through the lymphatic or blood system and subsequently colonize distant tissues and organs [111]. This process is known as tumour metastasis, and it accounts for 90% of cancer-associated mortality [112]. Metastatic cancer cells have acquired multiple genetic alterations that enable them to survive at a distant site. The processes involved in metastasis are quite complex because they entail different alterations that result in stimulation of angiogenesis, local invasion attachment, basement membrane disruption, matrix proteolysis and stimulation of growth factors, among others [113].

Angiogenesis is the formation of new blood vessels from pre-existing vessels to deliver nutrient and oxygen to a distant site, and it is critical for the colonization of secondary tumours. Saponins have been identified with the potential to inhibit the formation of new blood vessels in tumour cells [114]. For example, ginsenoside-Rb2, a dammarane saponin, slows down tumour metastasis of B16-BL6 by inhibiting tumour-induced angiogenesis [115]. During angiogenesis, endothelial cells migrate towards angiogenic signals to form capillary sprouts and tubes with a new basement membrane. Chan et al. highlighted that polyphyllin D suppresses the proliferation and migration of endothelial cells in *in vitro* experiment, in addition to the observation that saponins inhibited intersegmental vessel (ISV) formation in zebrafish similar to SU5416, a common vascular endothelial growth factor 2 inhibitor [116]. Similarly, *Panax notoginseng* restores defective ISV in zebrafish larva [117].

Yang et al. also reported that *Paris saponin* II (PSII) inhibited angiogenesis at low concentration in cancer cells and showed no toxicity to normal endothelial cells [118]. The anti-angiogenic activity was linked to the potential of PSII to modulate the expression of NF- κ B. By downregulating NF-

κ B expression, PSII reduced the activity of the downstream proteins such as VEGF, Bcl-2 and Bcl-xL. VEGF has been implicated in angiogenesis and lymphogenesis, and its activity is mediated by binding VEGF receptor (a tyrosine kinase receptor). Raddeanin A (an active triterpenoid saponin from the traditional Chinese medicinal herb *Anemone raddeana*) inhibits the phosphorylation of VEGFR2 by VEGF [119]. The authors further noted that RA binds to the ATP binding pocket of VEGFR2 and hinders its phosphorylation by VEGF, thereby preventing the activation of downstream effector proteins such as PLC γ 1, JAK2, FAK, Src, and Akt [119]. Additionally, sulphated saponin purified from sea cucumber can inhibit the phosphorylated form of VEGFR2 and the consequent downstream signalling pathway required for the mitogenic activity of VEGF in the endothelial cell [120].

Another mechanism through which saponin interferes with metastasis is by inhibiting cell adhesion molecules. Attachment of tumour cell to extracellular matrix (EM) and other similar cells is germane for metastasis. Different proteins such as integrins, CD44, ICAM and VLA-4 are responsible for cancer cells' cellular attachment [113]. *Paris polyphylla* can decrease the expression of intracellular adhesion molecule-1 (ICAM-1) in the cancer cell [121]. Furthermore, Huazhou et al. have also reported significant inhibition of vascular endothelial adhesion molecule-1 by saponin [122]. Likewise, a saponin monomer from dwarf lilyturf tuber inhibits hypoxia-induced integrin expression in the human breast cancer cell [123]. There is a minimal expression of adhesion molecule E-cadherin in cancer cells since E-cadherin mediates homotypic cell-cell interaction, which restricts cell mobility. Cancer cells undergoing metastasis show a lack of adhesion by inhibiting molecules such as E-cadherin required for cell-cell interaction. Molecules that increase E-cadherin expression impede tumour metastasis. The activity of E-cadherin is regulated by protein such as Cdc42 and Rac1 [111]. These proteins are Rho GTPases and their expressions are upregulated by saponins. For example, saponin fractions from *Asparagus officinalis* activate Cdc42

and Rac1 [111]. Furthermore, Ardipusilloside I also stimulate the activity of Rac1. By stimulating these upstream protein, saponins can inhibit cell migration [124].

Perhaps, one of the most studied deregulations in metastasis is tissue remodelling. It involves a family of proteins known as the matrix metalloproteinase (MMP). During metastasis, the tumour cell traverses the extracellular matrix (EM) barrier. This process is critical for cancer cell invasion, and it includes the proteolytic degradation of the EM by enzymes such as MMP2 and MMP9. Upregulation of MMP-2 and MMP-9 are particularly noted in cancer cells. By targeting multiple proteins participating in tissue remodelling pathways, saponins can significantly reduce cancer metastasis under *in vitro* and *in vivo* conditions [114, 125]. Several saponins have been identified with significant potential to specifically inhibit matrix degeneration protein such as MMP-2, vimentin and MMP-9 [125, 126]. In particular, ginsenoside Rd inhibits the expression of MMP-2, MMP-1 and MMP-7 [127].

Furthermore, epithelial-mesenchymal transition (EMT) proteins (MMP and MMP2) are regulated by NF- κ B and protein kinase such as MAPK, ERK, JNK, p38 and P13/AKT [128]. The inhibitory potential of specific saponins is linked to their ability to suppress the phosphorylation of some of these protein kinases and inhibit TNF- α induced NF- κ B activation [43, 129]. For example, kalopanaxaponin A, a triterpenoid saponin, inhibits the expression of MMP-9 in breast cancer cell by modulating P13/AKT and PKC pathways [130]. Ginseng saponin also inhibits MMP-9 in human astrogloma cell expression by suppressing activator protein-1 and MAPK [131]. In addition, trillium saponin downregulates MMP-2 and MMP-9 expression in HuH-7 cells [132].

The activity of matrix metalloproteinase can be further regulated by endogenous inhibitors, including tissue inhibitors of metalloproteinase (TIMP) and extracellular inducers of matrix metalloproteinase (EMMPRIN) [126, 133]. While the former works by reducing the activity of MMP, the latter stimulates the activity of MMP. Diosgenin, a steroidal saponin, inhibits EMMPRIN

and stimulate TIMP-2 expression in PC-3 cells [134]. Moreover, soybean saponins can stimulate TIMP-2 expression in colon cancer cells [133]. Similarly, Shuli et al. reported the upregulation of tumour cell TIMP-2 expression following *Rhizoma paridis* saponin treatment [129]. However, further studies are needed to understand MMP role in cancer and their regulation by saponins since it has been observed that increased TIMP-2 expression in glioblastoma patients is accompanied by severe adverse effects [135].

At the secondary site, tumour cells rapidly proliferate as a result of increased levels of growth factors. Different autocrine and paracrine growth factors such as bFGF, IGF-I and EGF are released by metastatic cells [113]. These growth factors have become therapeutic targets for certain anticancer drugs since their stimulation is essential for the rapid growth of cancer cell at a distant site [136]. Saponin DT13 can potentially block metastasis through the inhibition of tissue factor (TF) [123]. Timosaponin AIII suppresses hepatocyte growth factor-induced tumour invasion [37]. Zhuang et al. also reported that dihydro-diosgenin inhibited metastasis by suppressing endothelial cell-derived factor VIII and altering platelet function [137]. Beyond the direct role of the saponins on multiple pathways involved in metastasis, saponins can also be broken down in the body to yield secondary metabolites with potential anti-metastasis activity. For example, saponin metabolite from gut metabolism has shown significant metastasis inhibitory activity [138].

3.4 Saponin in Multidrug Resistant Cancer

The snag of drug-resistant remains a hurdle in the chemotherapeutic treatment of cancers. Several reports have documented the anticancer activity of saponins against drug-sensitive and drug-resistant cancer cell lines. Drug resistance in cancer is linked to several determinants, including increased tumour burden and metastasis, multiple chromosomal aberrations, physical barriers to chemotherapeutic agents, tumour micro-environment, adaptive cancer immune response and untargeted oncogenic drivers [139]. Saponins have been described that modulate some of the target

effectors such as pgp (p-glycoprotein) and Ras to elicit cytotoxic activity against resistant cancer cells. They have shown potent p-glycoprotein (an efflux pump highly expressed in many cancer resistant cells) inhibiting activity [140, 141]. Ras, an oncogenic driver in resistant cell lines, can be targeted using Paris saponin VII to inhibit colorectal cancer growth [142]. Some saponins have also demonstrated the ability to reverse multidrug-resistant in cancer cells and target angiogenesis in resistant cell lines [125, 141]. As a result of their potency to combat multidrug resistance, saponins are explored in combination therapy with other standard drugs to increase the therapeutic index of current anticancer regimens against drug-resistant cancer cells [143].

Another hurdle in cancer therapy is the elimination of cancer stem cells. These cells are capable of growing after effective treatment with chemotherapeutic agents [144]. Interestingly, saponins have shown inhibitory activity against cancer stem cell via a cell death mechanism involving the Wnt/ β -catenin signalling pathway [23].

4.0 Limitations and Prospects

The number of purified saponins with anticancer activity has increased significantly over the last two decades. Despite the widespread research and reports on the anticancer property of saponins and their derivatives, there are no FDA approved saponin-based anticancer drug [145]. Most of the studies describing the anticancer effect of saponins are from *in vitro* experiments, and only limited *in vivo* and clinical trial data are currently available. This limitation is a result of many factors ranging from drug-likeness property to toxicity index. There have also been concerns about the purity of natural saponins and their availability. This section considers the factors limiting the success of saponins as anticancer drugs and the future directions in cancer therapy.

Saponins possess a significantly high molecular weight (around 741 to 1808 Da) and a consequent high number of rotatable hydrogen bonds, total polar surface area and hydrogen bond donor and acceptor [145]. Generally, drugs with low molecular weight, high lipophilicity and fewer hydrogen

bond donors and acceptor are usually more bioavailable [146]. Saponin glycone has a notably lower oral bioavailability compared to aglycone saponin [147]. Furthermore, saponins such as *Panax notoginseng* can also be metabolized by some CYP450 isozymes, which might decrease the concentration of the active drug at the target site [148]. For several low orally bioavailable drugs, high dose oral administration or alternative route such as intravenous and intramuscular route are usually explored. However, intravenous administration of saponins is not a likely option to be explored since studies have shown that saponins possess high haemolytic activity, which may lead to anaemia [145]. The haemolytic activities of saponins are mediated by erythrocyte membrane permeabilization via interaction with the cholesterol of the plasma membrane [149, 150]. This activity is linked to critical carboxylic and hydroxyl functional groups of triterpenoid saponins [151].

The activity of saponins is dose-dependent, and a significant increase in oral dosage would mean a significant increase in bioavailability and action, which can significantly increase saponin toxicity [146]. Sub-acute, acute and chronic dose of saponins is associated with nephrotoxicity, hepatotoxicity and cardiotoxicity [152, 153]. There are, however, reports of non-toxic saponins even at higher concentration following oral administration in animal models [154, 155]. Besides, while several saponins are haemolytic, some of the saponins, including soya sapogenol, *Astragalus membranaceus* saponins, *Bupleurum chinense* saponins, are non-haemolytic [156, 157].

Structural optimization of saponin structure may prove to be very important in improving the drug-like property of saponins. Several anticancer drugs obtained from plants, such as paclitaxel, are structural derivatives of plant compounds. A detailed understanding of the structure-activity relationship of saponins would prove an invaluable tool to guide the development of bioavailable saponins as a potential anticancer drug candidate. Studies on QSAR of saponins to identify the functional groups responsible for the compounds haemolytic and cytotoxic activity have shown promising outcomes, in addition to structural modification to ensure selective action of the saponins.

For example, QSAR and QSPR studies of saponins isolated from *Pulsilla chinensis* showed that cytotoxic activity of the saponin was independent of its haemolytic activity. This technique, however, would help to identify the potent non-toxic drug candidate [149].

Targeted drug delivery is an alternative approach that could be explored further to increase the efficacy of saponin. Nanoparticles, due to their dimension, can evade clearance by plasma binding protein and reticuloendothelial system. By incorporating derived saponin compounds into nanocomposite, cytotoxic saponins can avoid clearance by plasma binding proteins (such as p-glycoproteins). Besides increasing the drug circulating time, nano-encapsulation limits the toxicity to normal cells. For instance, loading saponins into human serum albumin nanocomposites resulted in improved anticancer drug efficacy and no toxicity to healthy cells [158]. In addition, drug delivery vehicles involving micelles, self-assembled nano drugs, and liposomes can be functionalized with targeting moiety such as a cell-penetrating peptide to improve selectivity and reduce toxicity [159, 160].

Perhaps one of the most promising areas in saponin anticancer research is combination therapy. Saponins can be combined with other chemotherapeutic and radiotherapy treatment to improve efficacy and reduce toxicity. In combination with radiation treatment, Saponins induce apoptosis and cell cycle arrest in cancer cells [161, 162]. The saponin helped to sensitize resistant cancer cells to radiation treatment. This model can significantly treat tumour via a synergistic effect and reduces the side effects of chemotherapy in patients [33, 163]. Similarly, saponins have also been used as adjuvants to amplify the body's immune response to fight cancer [143, 148]. Saponins have likewise elicited synergistic effect leading to increased efficacy following co-administration with standard anticancer drugs [81, 164].

Concluding remark

The search for alternative therapeutic agents with increased efficacy and decreased toxicity is the crux of modern cancer research. Recent trends have unearthed the promising anticancer properties of an array of saponins using different *in vitro* and *in vivo* methods. The anticancer mechanism of saponin emanates from their ability to modulate a number of proteins required in the initiation, progression and invasion steps of cancer. The chemopreventive and chemotherapeutic action of saponins on cancer cells occurs via modulation of redox signalling, autophagy, apoptosis, necrosis, ferroptosis and cell cycle arrest. The anticancer activities of saponins are mainly dependent on their structure and ability to permeabilize the cancer cell membrane. However, the molecular structure of saponins poses specific challenges such as poor drug oral bioavailability, first-pass effect, and toxicity. Structural optimization, combination therapy, and conveying saponins through drug delivery vehicles (such as liposomes and nanoparticles) to target sites thus far show promising prospects that might enhance the development of saponins into standard drug in the nearest future.

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