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Review Article

A mechanistic and empirical review of antcins, a new class of phytosterols of formosan fungi origin

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ABSTRACT

Antcins are unique phytosterols isolated from *A. cinnamomea* and *A. salmomea*, which are the endemic fungus of Taiwan. *A. cinnamomea* has long been highly valued medicinal mushroom in Taiwan and traditionally used as a folk remedy for various human illness. Recent scientific explorations claimed that the pharmacological activities of *A. cinnamomea* and *A. salmomonea* are gone beyond their original usage. The therapeutic efficacy of these medicinal mushrooms was attributed to their high content of unique bioactive secondary metabolites, including terpenoids, benzenoids, ubiquinol derivatives, polysaccharides, lignans, nucleic acids, steroids, and maleic/succinic acid derivatives. Antcins is a group of steroids in *Antrodia* spp. with ergostane skeleton received much attention in Taiwan's academic circle due to their broad-spectrum of biological activities. At present, twelve antcins, *i.e.* antcin A, B, C, D, E, F, G, H, I, K, M, and N along with twelve derivatives/epimers (25*R*/*S*-antcin A, B, C, H, I and K) and seven analogs (methyl antcinate A, B, G, H, K, L and N) were identified. Several studies have demonstrated that antcins possessed anti-cancer, anti-inflammation, anti-oxidant, anti-diabetic, anti-aging, immunomodulation, hepatoprotection, and hypolipedimic activities. The main goal of this review is to define the chemistry, isolation, advances in production, and biological activities of antcins and their

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derivatives/analogs. Special attention has been given to a detail view of their biological activities *in vitro* and *in vivo* and their pharmacological potentials.

1. Introduction

Steroids are important constituents of cell membranes, which regulate membrane fluidity and permeability in plants. Steroids also play the functional role in fundamental biological processes such as signal transduction, cellular sorting, cytoskeleton reorganization, asymmetric growth, and infectious diseases. In contrast, triterpenoids are less regarded as essential component for normal growth and development, and although they largely exist in plants in simple unmodified form [1]. Especially, stigmasterol and sitosterol are 24-ethyl sterols, are main constituents of the steroids profiles of plants, which involved in the embryonic development of plants [2]. For instance, steroids have been recently shown to increase membrane cohesion in order to maintain plant membranes in a state of dynamics less sensitive to temperature shocks [3].

Ergosterol, a 5,7-diene oxysterol is the major sterols found in fungi, plays a functional role in growth and development, and is regarded as "fungal hormone" [4]. A recent study demonstrated that fungal pathogen-induced macrophage pyroptosis is triggered by fungal cell wall remodeling and exposure of glycosylated proteins in response to the macrophage phagosome. This was found that cell surface localization and/or total levels of ergosterols in the membrane correlated with the fungal pathogen induced pyrotopsis [5]. Due to their unique biosynthetic pathway, ergosterol biosynthesis is still the most applicable cellular pathway targeted by anti-fungal components [6]. Most of the current anti-fungal agents target ergosterols, either interrupting ergosterol biosynthesis or directly bind to cytoplasmic membrane bounded ergosterols. For example, azole compounds inhibit fungal cell wall synthesis through the inhibition of ergosterol biosynthesis, which is catalyzed by cytochrome P450 enzyme also known as 14α-demethylase during the conversion of lanosterol to ergosterol [7]. Not only synthetic agents, small molecules of plant inhibit growth and viability of *Candida albicans* through the inhibition of ergosterol biosynthesis [8]. On the other hand, amphotericin B, a polyene anti-fungal product preferentially bind to membrane ergosterols and creating transmembrane channels, resulting in an increased membrane damage, leakage of essential nutrients from the fungal cell and subsequent cell death [9]. Despite the fact, several ergosterol derivatives isolated from edible fungi, including *Antrodia cinnamomea*, *Antrodia salmonea*, *Grifola gargal*, *Ganoderma lucidum*, *Amauroderma rude*, *Polyporus umbellatus*, *Hericium erinaceus*, and *Pleurotus eryngii* exhibited anticancer, anti-inflammation, anti-diabetic, anti-aging, and anti-angiogenesis properties [10–16].

A. cinnamomea (Syn. *Antrodia camphorata* or *Taiwanofungus camphoratus*), is an unique medicinal mushroom of Taiwan and locally known as Niu-Chang-Chih, Chang-Chih, Niu-Chng-Ku, or Chang-Ku [17]. *A. cinnamomea* (Polyporaceae) is an endemic and expensive edible mushroom growing restrictedly on the inner cavity of its host species Bull Camphor tree, *Cinnamomum kanehirae* Hayata (Lauraceae) (Fig. 1A). Historically, *A. cinnamomea* was originally consumed by indigenous people of Taiwan as a traditional prescription for the treatment of liver complaints caused by alcohol intoxication. Being a local species, *A. cinnamomea* also used as a folk medicine for the treatment of various illness, including food poisoning, drug intoxication, abdominal pain, hypertension, diarrhea, skin irritation, liver and tumorigenic diseases. In modern Taiwanese culture, *A. cinnamomea* is believed to be a valuable gift from the heaven and it is claimed as the "National Treasure of Taiwan" as well as "Ruby of forest" [18,19]. Niu-Chang-Chih was first identified by Zhang and Su in 1990 as a new species under genus *Ganoderma* and named as *Ganoderma camphoratum* due to close resemblance with *G. lucidum* [20]. Later it was described as *A. cinnamomea*, and placed in genus *Antrodia*, because of its dimitic hyphae system with clamped generative hyphae and brown rot-causing ability [21]. After five years, a first phytochemical and bioactivity study of *A. cinnamomea* was reported by Chen and his coworkers [22]. He demonstrated that new steroids, including Zhankuic acid A, B and C were isolated from the

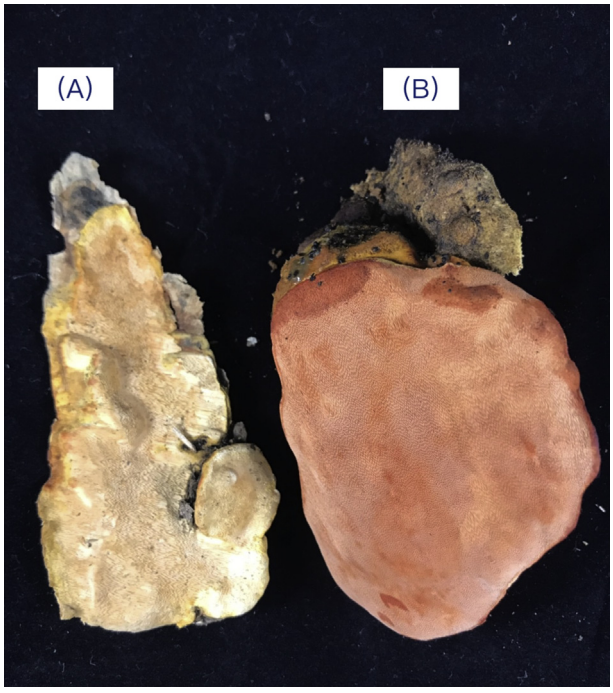


Fig. 1 – (A) Fruiting body of *A. salmonea* (salmon-pink). (B) Fruiting body of *A. cinnamomea* (orange colored).

fruiting bodies of *A. cinnamomea* and exhibited cytotoxicity to murine leukemia P-388 cells. Thereafter, *A. cinnamomea* received tremendous attention from academic and industrial researchers. Several recent reports revealed that *A. cinnamomea* possessed various biological activities including anticancer, anti-oxidant, anti-inflammatory, anti-diabetic, antimicrobial, anti-aging, hepatoprotective, neuroprotective, anti-hypertensive, anti-hyperlipidemic, and immunomodulatory properties [23–27].

A. salmonea T. T. Chang et W. N. Chou (Polyporaceae), was identified in 2004 as a new fungal species of the genus *Antrodia*. *A. solmonea* grown on hallow rotten trunk of its host species *Cunninghamia konishii* Hayata (Cupressaceae) (Fig. 1B). Both *A. salmonea* and *A. cinnamomea* are bitter in taste. The morphological features are almost identical, whereas the basidiome color of *A. salmonea* is salmon-pink and *A. cinnamomea* is cardinal red. Due to the practical difficulties of *A. cinnamomea* production and high market price, *A. salmonea* is often used as a substitute for *A. cinnamomea* [28]. *A. salmonea* also been used as a folk medicine for treating diarrhea, abdominal pain, hypertension, itchy skin and food, and alcohol detoxification [29]. Recent scientific studies have been reported that *A. salmonea* has various biological activities, including anti-inflammation [30], anti-oxidant [31], and anticancer [32]. These scientific studies have strongly supported that the pharmacological activities of *A. cinnamomea* and *A. salmonea* are go far beyond the original usage.

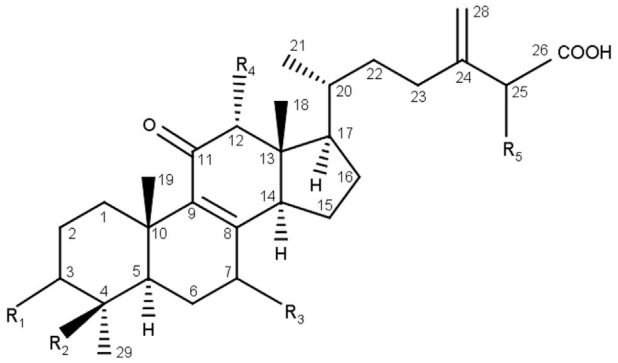
More than 160 compounds have been identified and structurally elucidated from *A. cinnamomea* and *A. salmonea* [28,33]. Among them 49 compounds are steroids, including 34 ergostanes and 10 lanostanes. The fruiting bodies of *A. cinnamomea* represent the major constituents and account for approximately 60% of dry weight [28,34]. In addition, it was found that the concentration of steroids are 10–30-folds higher in wild *A. cinnamomea* compared to *A. cinnamomea* produced by submerged culture [35]. The lanostanes were produced in both fruiting bodies and mycelia, whereas ergostanes were produced only in fruiting bodies, which are characterized as an important components for basidiomatal formation [36]. In 1995, Cherng and Chiang first isolated three ergosatane type phytosterols from the fruiting bodies of a newly identified fungal species *A. cinnamomea*, and these compounds were identified as antcin A, B, and C [37]. In parallel, Chen and Yang isolated another two ergostane type phytosterols, zhankuic acid B (antcin I) and zhankuic acid C (antcin H) along with a known compound zhankuic acid A (antcin B) from the fruiting bodies of *A. cinnamomea* [22]. In the later year, Cherng et al. [38] continued to isolate five new antcins, antcin D, antcin E, antcin F, methyl antcinate H, and methyl antcinate G from the fruiting bodies of *A. cinnamomea*. In the same period, Yang et al. [39] isolated two ergostane-type antcin analogs, methyl antcinate B (zhankuic acid D) and methyl antcinate H (zhankuic acid E) along with three lanostane-type steroids from the fruiting bodies of *A. cinnamomea*. Since than more than twenty ergostane-type steroids have been isolated and identified from the fruiting bodies and mycelia of *A. cinnamomea* and *A. salmonea*. The chemical structures of antcins, antcin analogs and their derivatives were illustrated in Fig. 2. The physio-chemical properties and source of the compounds were summarized in Table 1. These unique compounds were only distributed in *A. cinnamomea* and *A. salmonea*.

Given the phytochemical and pharmacological significance of antcins and its emergence as the potential therapeutic compounds, a comprehensive review ranging from their biosynthesis to therapeutic implementation has become a prime requirement. The present review is aimed to compile the mechanistic and empirical view of the chemistry, biosynthesis, and pharmacological properties of antcins.

2. Chemistry and biosynthesis of antcins

Steroids and triterpenoids are biogenetically derived from acetate/mevalonate pathway in cytosol and endoplasmic reticulum of the plant cell. Isopentenyl pyrophosphate (IPP) serves as the fundamental building block for the biosynthesis of all the terpenoids, including steroids which are subsequently derived from triterpenoids [40]. The conversion of IPP into dimethylallyl diphosphate (DMAPP) serve as the primer for elongation through prenyl transferase. Then, the farnesyl pyrophosphate (FPP) are formed from the metabolites by farnesyl pyrophosphate synthase, which is the branch point for synthesis of squalene or related acyclic C_{30} precursors of triterpenoids and steroids. The last common intermediate of both steroids and triterpenoid pathway is 2,3-oxidosqualene [1]. Both steroids and triterpenes are made up of a tetracyclic cyclopenta[α]phenanthrene ring and a long flexible side chain at the C_{17} carbon atom. Cyclization of 2,3-oxidosqualene is catalyzed by enzymes known as 2,3-oxidosqualene catalases enable the formation of more than 20,000 different structures of triterpenes or steroids [41,42]. The most common structures of triterpenoids, including acyclic, mono-, bi-, tri-, tetra-, and pentacyclic triterpenoids. The most studied type of triterpenoids and steroids are tetracyclic derivatives such as lanostane, ergostane, cucurbitane, protostane, euphane, cycloartane, dammarane, tirucallane and apoptirucallane, and pentacyclin derivatives including adianane, baccharane, bauerane, ernane, filicane, friedelane, gammacerane, glutinane, hopane, lupine, multiflorane, neohopane, oleanane, pachysanane, taraxastene, taraxerane, and ursane [43]. The diverse use of triterpenoids in the phytochemical and pharmaceutical industries have distinguish them as economically important substances. Recent *in vitro* and *in vivo* studies revealed their multidirectional properties [41,44–46].

Direction in to the steroids pathway, the specialized metabolites are synthesized via the chair-boat-chair (CBC) conformation. In plants with photosynthetic apparatus, an enzymatic steps involved in conversion of squalene oxide into cycloartenol and cucurbitadienol by cycloartenol synthase (CAS) and cucurbitadienol synthase (CPQ), respectively. Whereas, non-photosynthetic fungi converts squalene oxide into lanosterols by lanosterol synthase (LAS). Lanosterol serves as a precursor of steroids such as ergosterol in fungi and cholesterol in mammals. The enzymatic conversion of lanosterol into pathway end-products, cholesterol and ergosterols implies several demethylation steps at C_{14} and C_4 positions, which appear to be pre-requisite for a further transmethylation on C_{24}. It was identified that 19 enzymatic steps involved in conversion of lanosterol to cholesterol in



No	Compound	R1	R2	R3	R4	R5	R6
1/2	25*R*/*S*-antcin A	=O	H	-	H	α-CH_3/β-CH_3	
3/4	25*R*/*S*-antcin B	=O	H	=O	H	α-CH_3/β-CH_3	
5/6	25*R*/*S*-antcin C	=O	H	α-OH	H	α-CH_3/β-CH_3	
7	Antcin D	=O	H	=O	H	CH_3	
8	Antcin E	=O	H	-	H	CH_3	
9	Antcin F	=O	H	α-OH	H	CH_3	
10	Antcin G	=O	H	OAc	β-OH	CH_3	
11/12	25*R*/*S*-antcin H	β-OH	H	=O	β-OH	α-CH_3/β-CH_3	
13/14	25*R*/*S*-antcin I	β-OH	H	=O	H	α-CH_3/β-CH_3	
15/16	25*R*/*S*-antcin K	β-OH	OH	α-OH	H	α-CH_3/β-CH_3	
17	Antcin M	β-OH	-	-	β-OH	CH_3	
18	Antcin N	α-OH	-	β-OH	α-OH	CH_3	
19/20	25*R*/*S*-Methyl antcinate A	=O	H	-	H	α-CH_3/β-CH_3	CH_3
21/22	25*R*/*S*-Methyl antcinate B	=O	H	=O	H	α-CH_3/β-CH_3	CH_3
23	Methyl antcinate G	=O	H	OAc	β-OH	CH_3	CH_3
24/25	25*R*/*S*-Methyl antcinate H	β-OH	H	=O	β-OH	α-CH_3/β-CH_3	CH_3
26/27	25*R*/*S*-Methyl antcinate K	β-OH	OH	α-OH	H	α-CH_3/β-CH_3	CH_3
28	Methyl antcinate L	α-OH	-	α-OH	α-OH	CH_3	CH_3
29	Methyl antcinate N	α-OH	-	β-OH	α-OH	CH_3	CH_3

Fig. 2 – Chemical structures of antcins and their derivatives and analogs (1–29) derived from *A. cinnamomea* and *A. salmonea*.

mammals begins with C_{14}-demethylation and is followed by C_{14}-reduction, C_4-demethylation, C_{24}-reduction, C_5-desaturation and C_7- reduction [47]. The structure of ergosterol differs from that of cholesterol by the presence of additional double bonds, on the B-ring and on the acyl chain [48]. Also, a methyl group is present in C_{24}.

In ergosterol biosynthesis, lanosterol 14α-demethylase (Erg11p, a member of the CYP51 class of the cytochrome P450 enzymes) catalyzed 14α demethylation of lanosterol to form 4,4-dimethyl-zymosterol, which is the first and rate-limiting step in ergosterol biosynthesis in fungi (Fig. 3). The second step in erosterol biosynthesis is conversion of 4,4-dimethyl-zymosterol into zymosterol by C_4 sterol methyl oxidase (Erg25p), C_3 sterol dehydrogenase (Erg26p) and 3-keto reductase (Erg27p). Next, sterol 24-C-methyl transferase (Erg6p) converts zymosterol to fecosterol by methylating position C_{24}. Sterol D^8-D^7 isomerase (Erg2p) catalyzes the conversion of fecosterol to episterol. The second last step in the ergosterol biosynthesis is addition of double bond at the C_5 carbon of the B-ring of episterol by sterol $\Delta^{5,6}$-desaturase (Erg3p) to produce ergosta-5,7,24(28)-trienol. The final step is conversion of ergosta-5,7,24(28)-trienol into ergosta-5,7,22,24(28)-tetraenol by sterol Δ^{22}-desaturase (Erg5p) and conversion of ergosta-5,7,22,24(28)-tetraenol into ergosterol by sterol Δ^{24}-reductase (Erg4p) [1,4,49].

3. Pharmacological properties antcins

During the last three decades, *A. cinnamomea* has been one of the most widely studied higher basidiomycetes due to its variety of therapeutic and medicinal properties. Indeed, antcins, a class of ergostane-type steroids from *A. cinnamomea* or *A. salmonea* claimed substantial therapeutic values. In this section, we summarized the bioactivities of antcins according to the published reports.

3.1. *Anti-cancer effects of antcins*

Cancer is the second leading cause of death worldwide, surpassed by cardiovascular diseases. International Agency for Research on Cancer (IARC), a part of the World Health Organization (WHO) estimated that cancer incidence dramatically increased to 18.1 million new cases and 9.6 million cancer

Table 1 – Physico-chemical properties of antcins and their analogs.

Compounds	Extraction methods	Identification methods	Color & appearance	Melting point	Mol. formula & Mol. weight	Reference
Antcin A (4α-methylergost-8,4(8)-diene-3,11-dion-26-oic acid)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR	Color less crystals	173-175 °C	$C_{29}H_{42}O_4$ 454.30	[37,153]
Antcin B (Zhankuic acid A) (4α-methylergosta-8, 24(28)-diene-3,7,11-trion-26-oic acid)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR	Pale yellow crystals	136-138 °C	$C_{29}H_{40}O_5$ 468.28	[22,37]
Antcin C (7β-hydroxy-4α-methylergost-8, 4(8)-diene-3,11-dion-26-oic acid)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR	White crystals	187-189 °C	$C_{29}H_{42}O_5$ 470.30	[37]
Antcin D (Zhankuic acid F) (14α-hydroxy-4α-methylergosta-8,24(28)-diene-3,7,1 1 -trione-26-oic acid)	MeOH-FCBAC	^{1}H NMR, ^{13}C NMR, UV, IR, DEPT, HR-EI-MS, EI-MS	Pale yellow crystals	155-157 °C	$C_{29}H_{40}O_6$ 484.28	[38,154]
Antcin E (3,11-dioxo-4α-methylergost-8,14,24(28)-trien-26-oic acid)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR, HRMS	Yellow liquid	–	$C_{29}H_{40}O_6$ 452.29	[38]
Antcin F (3,11-dioxo-7β-hydroxy-4α-methylergost-8,14,24(28)trien-26-oic acid)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR, HRMS	Colorless crystals	–	$C_{29}H_{40}O_5$ 468.28	[38]
Antcin H (Zhankuic acid C) (3α,12α-dihydroxy-4α-methylergosta-8,24(28)-dien-7,LL-dione-26-oic acid)	EtOAc-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR	Pale yellow crystals	–	$C_{29}H_{40}O_5$ 468.28	[22]
Antcin I (Zhankuic acid B) (3α-hydroxy-4α-methylergosta-8,24(28)-diene-7,11-dion-26-oic acid)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, APCI-MS	Pale yellow crystals	187-190 °C	$C_{29}H_{42}O_5$ 470.30	[22,153]
Antcin K (3α,4β,7β-trihydroxy-4α-methylergosta-8,24(28)-dien-11-on-26-oic acid)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, ESI-MS, HRMS	Colorless crystals	227-230 °C	$C_{29}H_{44}O_6$ 488.31	[153]
Antcin M (3α,12α-dihydroxy-4α-methylergosta-8,24(29)-dien-11-0n-26-oic acid)	MeOH-ASFB	^{1}H NMR,^{13}C NMR, ESI-MS	Yellow solid	93-95 °C	$C_{26}H_{44}O_5$ 472.31	[30]
Methyl antcinate A (methyl 3,11-dioxo-4α-methylergosta-8,24(28)-dien–26-oate)	MeOH-ACFB	^{1}H NMR,^{13}C NMR, ESI-MS	Colorless crystals	–	$C_{30}H_{44}O_4$ 468.32	[62]
Methyl antcinate B (Zhankuic acid D) (methyl 4α-methylergosta-8,24(28)-diene-3,7,11-trion-26-oate)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR	Pale yellow crystals	–	$C_{31}H_{45}O_5$ 496.1	[39,153]
Methyl antcinate G (methyl 7α-acetoxy-3,11-dioxo-4α-methylergost-8,24(28)-dien-26-oate)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR, HRMS	Colorless liquid	–	$C_{32}H_{46}O_6$ 526.32	[38]
Methyl antcinate H (Zhankuic acid E) (methyl 3α,12α-dihydroxy-4α-methylergosta-8,24(28)-diene-7,11-dion-26-oate)	MeOH-ACFB	^{1}H NMR, ^{13}C NMR, UV, IR, DEPT, EI-MS	Pale yellow crystals	170-173 °C	$C_{31}H_{47}O_6$ 500.31	[38,39]
Methyl antcinate K (Methyl 3α,4β,7β-trihydroxy-4α-methylergosta-8,24(29)-dien-11-on-26-oic acid)	MeOH-FBAS	^{1}H NMR,^{13}C NMR,EI-MS	White plates	195-197 °C	$C_{30}H_{46}O_6$ 502.32	[30]
Methyl antcinate L (Methyl 3α,7α,12α-trihydroxy-4α-methylergosta-8,24(29)-dien-11-on-26-oate)	MeOH-FBAS	^{1}H NMR, ^{13}C NMR,EI-MS	White powder	205-206 °C	$C_{30}H_{46}O_6$ 502.33	[30]

ASFB, *A. cinnamomea* fruiting bodies; FCBAC, Fermented culture broth of *A. cinnamomea*; ASFB, *A. salmonea* fruiting bodies.

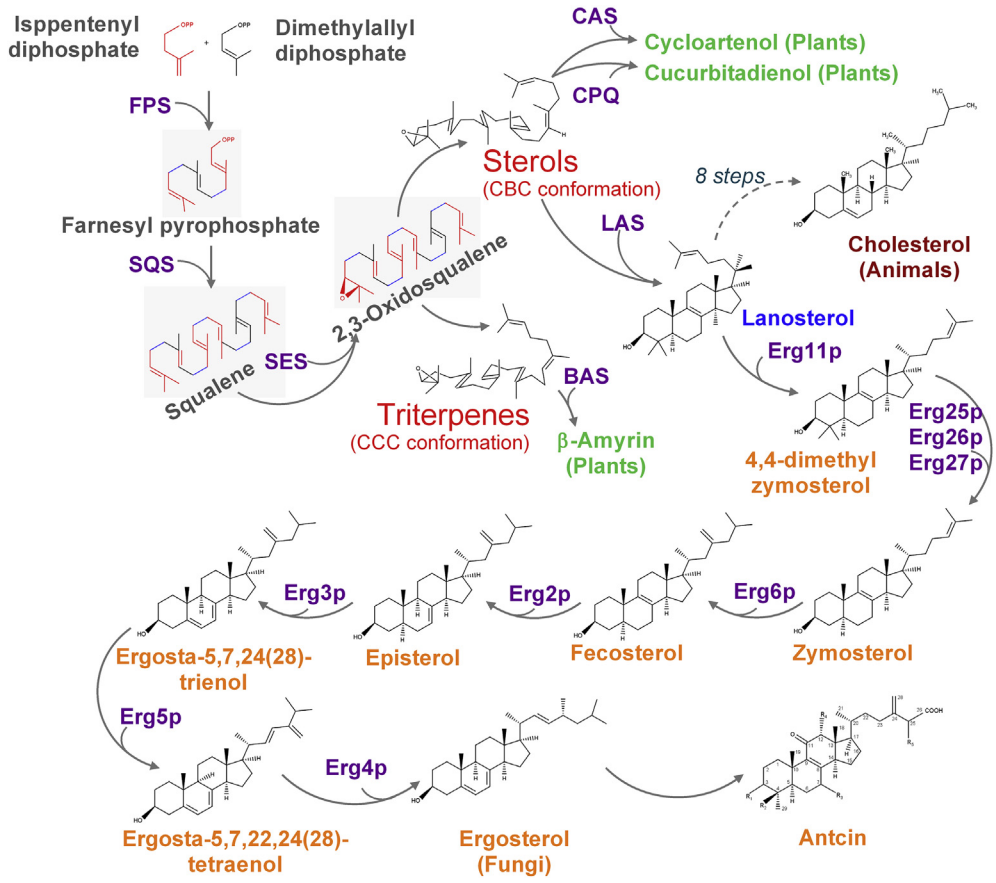


Fig. 3 – Biosynthetic pathway of antcins. The enzymes that catalyze the various steps are indicated in purple colored font. Enzyme abbreviations: FPS, farnesyl pyrophosphate synthase; SQS, squalene synthase; SQE, squalene monooxygenase or epoxidase; SHC, squalene-hopene cyclase; LAS, lanosterol synthase; CAS, cycloartenol synthase; CPQ, cucurbitadienol synthase; BAS, β-amyrin synthase. Erg2p, sterol D^8-D^7-isomerase; Erg3p, sterol $D^{5,6}$-desaturase; Erg4p, sterol C-24(28) reductase; Erg5p, sterol C-22 desaturase; Erg6p, sterol 24-C-methyl transferase; Erg11p, lanosterol C-14 demthylase; Erg25p, C-4 sterol methyl oxidase; Erg26p, sterol-4α-carboxylate-3-dehydrogenase; Erg27p, 3-keto reductase. Other abbreviations: CBC, chair-boat-chair; CCC, chair-chair-chair.

death in 2018 [50]. Cancer is a generic term for a large group of diseases characterized by alterations in cell physiology, such as uncontrolled replication potential, self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, escape from programmed (apoptosis) or non-programmed cell death (autophagy), sustained neo-vasculature (angiogenesis) and migration and tissue invasion [51]. The treatments for cancer can be generally divided into chemotherapy, radiation therapy, surgery, targeted therapy, gene therapy, immunotherapy, hormone therapy, cryotherapy, hyperthermia, angiogenesis inhibitor therapy, traditional medical practice, and other palliative methods [52]. Among them, chemotherapy is the most common treatment modality. Natural products are the most promising anti-cancer chemotherapeutic or chemo-preventive agents derived from plants and microbes of terrestrial or marine origin [53]. There are three-quarters of anti-cancer agents approved and pre-NDA candidates are either natural or derived from natural products, such as paclitaxel, docetaxel, vinblastine, vincristine, etoposide, topotecan, and irinotecan are among the active currently available chemotherapeutic agents [54]. It has been claimed that chemotherapeutic agents derived from natural products are more effective for cancer patients than those of synthetic origin [55]. The signaling pathways leading to anti-cancer properties of natural products have been widely studied, including inhibition of pathways involved in tumor growth (*e.g.* tyrosine kinases and epidermal growth factors), induction of apoptosis by mitochondrial-dependent (intrinsic) and death receptor-associated (extrinsic) pathways, inhibition of key enzymes involved in signal transduction (eg. proteases), inhibition of extracellular matrix degradation, epithelial-to-mesenchymal transition (EMT) and angiogenesis [54]. The anti-cancer properties of *A. cinnamomea* and *A. salmonea* were extensively studied and reviewed [18,25,26,33]. In this review we emphasize the anti-cancer properties of antcins and their derivatives and analogs. Fig. 4 summarizes detailed molecular mechanisms involved in antcins-mediated anti-cancer properties.

AntB (**3/4**), AntH (**11/12**) and AntI (**13/14**) were isolated from the fruiting bodies of *A. cinnamomea* and their cytotoxic effect was evaluated in murine lymphocytic leukemia tumor cell line, P-388. AntB (**3/4**) and AntI (**13/14**) exhibited cytotoxicity to P-388 cell line with an IC_{50} value to 1.8 and 5.4 μg/mL, respectively [22]. Four ergostane steroids, AntB (**3/4**), AntC (**5/**

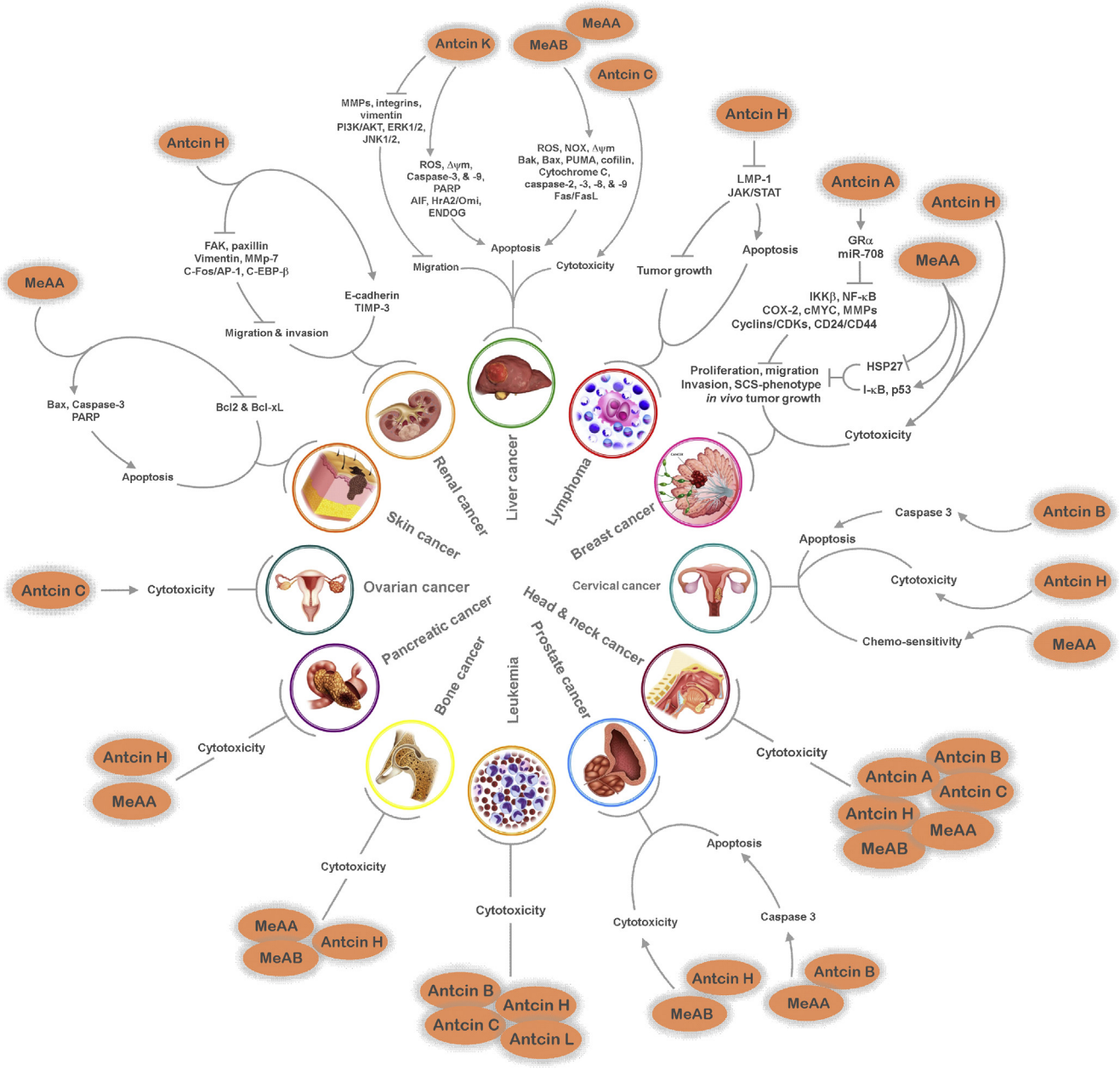


Fig. 4 – Molecular targets of antcins against various human cancers.

6), AntH (11/12) and AntK (15/16) and one lanostane steroids, dehydroeburicoic acid, were isolated from the steroids-rich fraction of wild-collected *A. cinnamomea* were investigated on their cytotoxic activity against human leukemia HL60 cells [56]. Regarding the cytotoxic properties of isolated Antcins in HL60 cells, AntB (3/4), AntC (5/6), and AntH (11/12) exhibited cytotoxicity to HL60 cells, whereas AntK (15/16) does not show cytotoxicity even at a high concentration (100 μg/mL). However, the distinct cytotoxicity was observed in dehydroebruicoic acid treated cells. Further annexin V/propedium iodide apoptosis assay revealed that AntB and dehydroebuicoic acid increased the apoptotic population of HL60 cells.

Epstein–Barr virus (EBV) is a wide-spread human γ-herpes virus; also, a first identified human virus with a proven association with several human malignancies. It has been demonstrated that in human, EBV infection leads to the development of B-cell lymphomas [57]. Latent membrane protein-1 (LMP-1), the principle viral oncoprotein and a member of TNF-α receptor superfamily contributes to the proliferation of EBV-infected primary human B lymphocytes *via* the JAK/STAT signaling pathway [58]. Therefore, the chemotherapeutic agent, which targets LMP1-induced JAK/STAT signaling is a proven strategy for effective treatment of B-cell lymphomas. It has been shown that AntH (11/12) induce apoptosis in human lymphoma cell line through the suppression of LMP-1-mediated JAK/STAT signaling cascade [59]. Also, AntH enhances chemo-sensitivity to a low-dose of methotrexate in lymphoma cells. Furthermore, treatment of AntH with low-dose methotrexate significantly reduced

tumor growth and increased the survival rate of tumor-bearing mice. This study suggests that AntH could be a potential therapeutic agent for the treatment of EBV-infected B-lymphoma. The following study by Lai et al. [60] further extended the anti-cancer properties of AntK (**15/16**). Incubation of Hep3B cells with AntK (80–125 μM) significantly reduced cell viability within 48 h. The reduction in cell viability was reasoned by the induction of apoptosis. Further, in-depth investigation of the molecular mechanisms revealed that AntK triggers ROS generation and adenosine triphosphate depletion, leading to endoplasmic reticulum stress and resulting in mitochondrial membrane permeability as indicated by the increase of mitochondria-dependent pro-apoptotic proteins such as Bak and Bax were observed. After losing the mitochondrial membrane potential, caspase-independent and caspase-dependent apoptosis-related proteins, including HtrA2/Omi, apoptotic-induced factor (AIF), endonuclease G, and cytochrome C were released into the cytoplasm. AntK-mediated release of cytochrome C further activates caspase-9 and caspase-3 and PARP, ultimately leading to cell apoptosis.

Du et al. [35] investigated there is an existing relationship between the stereochemistry and anti-cancer properties of 25(*R*) and 25(*S*) ergostane isomers in a panel of human leukemia cancer cells lines, CCRF-CEM, Molt 4 and HL60. AntB and AntC derivatives with carbonyl functional group at C_3 position exhibited better cytotoxicity in leukemia cells with IC_{50} ranging from 16.44 to 77.04 μg/mL. They also demonstrated that compounds with a hydroxyl group at C_3 position were less cytotoxicity (IC_{50} > 80 μg/mL) to leukemia cells, suggesting the presence of a carbonyl group is vital for their bio-activity. Besides, the presence of other hydroxyl groups in ring C_4, C_7, and C_{12} positions of ring A, B, and C did not show significant cytotoxicity. Next, the correlation between sterioconfiguration of antcins and their cytotoxicity was explained by that 25(*R*) and 25(*S*) isomers of AntB (**3/4**) showed higher cytotoxic effect than that of their mixture form, AntB (**3/4**) in all tested cells. On the other hand, 25(*R*) and 25(*S*) isomers of AntC (**5/6**) lost their cytotoxic potency when compared with mixture form (IC_{50} > 80 μg/mL) in HL60 cells, whereas in Molt4 cell line, 25(*R*) from of AntB (**3/4**) and AntH (**3/4**) were cytotoxic than the related 25(*S*) form. In CCRF-CEM cells, the AntB epimers, 25(*R*)-AntB (**3**) and 25(*S*)-AntB (**4**) and AntB (**3/4**) exhibited nearly equal cytotoxic effect. This study does not clearly define a cross-link between sterioconfiguration and cytotoxic effect of Ants. In parallel, we found that 25(*S*) isomer of AntC (**5/6**) presented cytotoxicity to human hepatoma HepG2 and breast carcinoma MCF-7 cell lines with an IC_{50} value of 14.5 and 12.8 μg/mL, respectively. When compared to 25(*S*) isomer of AntC (**5**), 25(*R*) isomer of AntC (**6**) did not showed significant cytotoxicity to both of the cell lines. This data specified that sterioconfiguration at C_{25} position for AntC is playing a crucial role for its cytotoxicity [61]. However, further bio-functional analysis are highly warranted to explain this phenomenon.

MeAA (**19/20**), an analog of AntA (**1/2**) isolated from the chloroform extracts of fruiting bodies of *A. cinnamomea* exhibited cytotoxic activity against human oral squamous cell carcinoma cell lines, OC-2 and OEC-M1 with 50% reduction in cell survival at 37.4 and 24.5 μM, respectively [62]. Further annexin V/PI-FITC analysis with flow cytometry revealed that treatment with MeAA induced apoptosis as indicated by the percentage of annexin V-positive cells were increased from 0.69% to 59.73% and 62.33% in OEC-M1 cells and from 0.67% to 15.35% and 9.09% in OC-2 cells with 25 and 50 μM of MeAA, respectively. Besides, increased expression of pro-apoptotic marker Bax, activation of caspase-3 and PARP cleavage, and decreased anti-apoptotic proteins, Bcl-2 and Bcl-xL. Indeed, the cytotoxic effects of MeAA was different in these two cell lines that OEC-M1 was more sensitive to MeAA than that of OC-2 cells, whereas exposure of normal gingival fibroblasts to MeAA for 24 h did not induce cytotoxicity up to a maximum concentration of 50 μM, suggesting a selective cytotoxic activity of MeAA on tumor cells. In parallel, the same research group was involved to identify a potent cytotoxic candidate, the apoptotic effect of AntA (**1/2**), AntC (**5/6**), and MeAA (**19/20**) against human hepatocellular carcinoma cell lines, HepG2, Hep3B, and Huh-7 were investigated [63]. Hepatoma cells were exposed to various concentrations of AntA (5–200 μM), AntB (50–200 μM) and MeAA (5–150 μM) for 24 h and a result of tryphan exclusion assay found that MeAA exhibited potent cytototxic activity against HepG2, Hep3B and Huh-7 cells with IC_{50} value of 52.2, 78.0 and 30.2 μM, respectively. Treatment with 100 μM of MeAA induced apoptosis in Huh-7 cells which was manifested by increase of caspase-3 activity, DNA fragmentation, sub-G1 population and TUNEL-positive cells. The underlying molecular mechanisms were revealed by that MeAA triggered the intrinsic (mitochondria-dependent) apoptotic pathway, as indicated by decrease of anti-apoptotic proteins, Bcl-2 and Blc-xL, and increase of pro-apoptotic proteins, Bax, Bak and PUMA, and mitochondrial membrane potential, release of cytochrome *C*, as well as caspase-2, -3 and -9 activation. In addition, MeAA induced intracellular ROS production and NADPH oxidase activity in Huh-7 cells. The same group continued their investigation on cytotoxic activity of AntB (**3/4**) and MeAB (**21/22**) on HepG2 cells [64]. It was found that both MeAB and AntB exhibited cytotoxicity to HepG2 cells with IC_{50} 25.8 and 38.4 μM, respectively. This effect was reasoned by induction of apoptosis, as characterized by a sequential event of loss of mitochondrial membrane potential, release of cytochrome *C*, activation of caspase-2, -3, -8 and -9, chromatin condensation, DNA fragmentation and increase of sub-G1 population. In this study, they also revealed that MeAB and AntB induce Fas/FasL-dependent extrinsic (death receptor) pathway of apoptosis, as evidenced by Fas antagonist antibody blocked MeAB or AntB-induced apoptosis in HepG2 cells. Results also signify that MeAB (**21/22**) and AntB (**3/4**) induced free radicals (H_2O_2) that triggered apoptosis followed by loss of mitochondrial membrane potential, because the apoptosis was inhibited by pre-treatment with antioxidants (ascorbic acid and *N*-acetyl cysteine) or free radical scavenger enzymes, superoxide dismutase (SOD) and catalase (CAT). The use of MeAB and AntB provoked NADPH oxidase activity, a source of ROS generation that was abolished by diphenylamine, an inhibitor of NADPH oxidase. This study provided the pivotal role of MeAB and AntB (**3/4**) on oxidative stress-mediated intrinsic and extrinsic apoptotic pathways in human hepatocarcinoma cells.

The further mechanistic study revealed that MeAA (**19/20**) induced translocation of cofilin from the cytosol to

mitochondria, thereby affecting the mitochondrial membrane potential, which was confirmed by that pre-treatment of cyclosporine and bongkrekic acid, inhibitors of mitochondrial membrane potential blocked MeAA activity. On the other hand, pre-treatment with ascorbic acid and *N*-acetyl cysteine, are known as antioxidants attenuated MeAA-induced apoptosis in Huh-7 cells. This study provided a new insight that activation of ROS-dependent cofilin and Bax-triggered disruption of mitochondrial membrane potential are the critical mechanisms of MeAA-induced apoptosis in human hepatocarcinoma cells.

The cytotoxicity and effects on apoptosis of antcins and their analogs were reported in various cancer cell lines, including head and neck (TSCCa, GNM, KB, OC-2 and OEC-M1), pancreas (Panc-1 and AsPC), breast (BT474 and T47D), prostate (PC-3), ovary (OVCAR-3), cervix (HeLa), gastric (AGS), and bone (U2-OS) [65]. MeAA (**19/20**) exhibited potent cytotoxicity to almost all the tested cell lines with IC_{50}/CC_{50} was ranging from 12.6 to 119.5 μM. They used the prostate cancer cell line, PC-3 to study the mechanism of cell death and found that MeAA-induced cell death was reasoned by apoptosis as indicated by increased caspase-3 activity. MeAB (**21/22**) expressed cytotoxicity to GNM, KB, OC-2, OEC-M1, PC-3, and U2-OS cell lines. Next to MeAA (**19/20**), AntH (**11/12**) exhibited cytotoxicity to a wide-range of cancer cells, GNM, KB, AsPC, T47D, PC-3, HeLa and U2-OS with an IC_{50}/CC_{50} ranging from 13.7 to 177.0 μM. AntB (**3/4**) showed cytotoxicity and the IC_{50} were 187.5, 167.4, and 165.5 μM to GNM, PC-3, and HeLa cells, respectively. AntC (**5/6**) reduced 50% of viable in KB and OVCAR-3 cells at a concentration of 124.0 and 144.8 μM, respectively. Interestingly, AntA (**1/2**) showed selective cytotoxicity to head and neck cancer cells that TSCCa, GNM, and KB cells lost their 50% survival rate at a concentration of 188.1, 87.6, and 179.6 μM, respectively. Also, caspase-3 activity assay revealed that AntB (**3/4**) and AntH-mediated reduction in cell viability/cytotoxicity was caused by induction of cellular apoptosis, as evidenced by the increased amount of intracellular caspase-3 in AntB (**3/4**) and AntH (**11/12**) treated cells.

A self-renewable and extensively proliferative stem cells, also known as cancer stem cells (CSCs) are believed to be responsible for the initial formation and progression of cancer, as well as the reoccurrence of cancer after treatment, due to the fact that CSCs are more resistant to conventional treatment than differentiated cancer cells [66]. Based on their unique characteristic features and dynamics CSCs can be targeted by several strategies, such as inhibition of self-renewal, induction of differentiation into mature cancer cells, and sensitization to anti-cancer agents [67]. Over a decade, several dietary phytochemicals have been reported to intervene various signaling pathways, which are involved in stemness maintenance of CSCs or to modulate the CSC phenotype and promote sensitization to conventional chemotherapeutic agents [67–69]. In a case of breast cancer, MeAA (**19/20**) displayed low cytotoxicity towards MCF-7 cells (IC_{50} > 50 μM). Indeed, treatment with MeAA suppressed the cancer stem cell (CSCs) phenotype of MCF-7 cells. Mammosphere culture has been used to enrich CSCs in MCF-7 cells [70]. Result of mamospheres analysis has shown that treatment with MeAA suppressed the formation mamaospheres of MCF-7 cells [71]. The low concentration of MeAA 10 μM reduced only the secondary spheres, whereas 50 μM of MeAA markedly blocked both primary and secondary spheres. MeAA also displayed anti-migration property as indicated by delayed wound closure of MCF-7 sphere cells. It has been demonstrated that heat shock protein 27 (Hsp27) could regulate the maintenance of breast CSCs through EMT and nuclear factor-κB (NF-κB). Further immunoblotting analysis revealed that MeAA down-regulated Hsp27 and upregulated inhibitor-κB (I-κB) expression, a negative regulator of NF-κB in MCF-7 sphere cells. In addition, MeAA increased the expression level of p53, a known tumor suppressor has been characterized to block the self-renewal of breast CSCs [72].

Metastasis is characterized as the final and most devastating stage of malignancies and is responsible for as much as 90% cancer-related death [73]. The dissemination of cancer cells from primary tumors and subsequent journey to the secondary site are remarkable if considered on a cell's scale of magnitude [74]. Chaffer and Weinberg [73] suggested that the complex metastatic cascade can be conceptually organized and simplified into two major phases: (i) journey of a cancer cell from the primary tumor to the microenvironment of a distant organ and then (ii) colonization, the formation of a macroscopic second tumor. The initial step or phase I, involved several physiological events, including loss of cell polarity, detachment from the surrounding cells in the primary tumor, increased interaction with extracellular matrix through integrin and migration through ECM towards bloodstream and lymphatic vessels. This process is indicated as epithelial-to-mesenchymal transition and has been extensively studied as an initial event of tumor cell metastasis [75]. Chemotherapy is used as a main-stream treatment form many types of metastatic cancers; however, severe side effects may be brought at the effective dose of the chemotherapeutic agents [76]. Therefore, in addition to minimizing the growth of the existing tumor or tumor relapse, treatments that limit its metastasis have been shown to enhance the survival of cancer patients. AntK isolated from the basswood cultivated *A. cinnamomea* exerts anti-metastatic properties in human hepatoma Hep3B cells [77]. Result of this study demonstrated that AntK (**15/16**) inhibits hepatoma cell migration and invasion potentially through down-regulation of matrixmettalloprotenase, MMP-2, and MMP-9 activity. In addition, AntK regulates the epithelial-to-mesenchymal transition by up-regulation of epithelial marker E-cadherin and down-regulation of mesenchymal marker vimentin. Further in-depth investigation for the molecular mechanism revealed that AntK down-regulate the protein expression levels of integrin-β1, -β3, -α5 and αv and reduced the phosphorylation of Src, PI3K, AKT, MEK1/2, ERK1/2 and JNK1/2 at Tyr^{416}, Tyr^{458}, Ser^{473}, Ser^{217}/Ser^{221}, Thr^{202}/Tyr^{204}, and Thr^{183}/Tyr^{185} residues, respectively. These results imply that AntK could be a promising agent to inhibit the metastasis of human liver cancer cells. Metastasis has been considered as a poor prognostic factor in human renal cell carcinoma (RCC); therefore, developing safe and effective therapeutic agents for the treatment of metastatic RCC is urgently required [78]. Treatment with AntH (**11/12**) has shown to have a preventive effect on human renal cell carcinoma migration and invasion [79]. AntH at low concentrations (20–100 μM) caused growth inhibitory effect but did not display any sign of cytotoxicity by

morphological analysis. The inhibitory effect of AntH on migration and invasion of RCC were accompanied by the decrease of FAK and paxillin phosphorylation, impairment of lamellipodium formation, up-regulation E-cadherin and TIMP-3 and down-regulation of vimentin and MMP-7 in RCCs. Further analysis revealed that AntH suppressed transcriptional activity of c-Fos/AP-1 and C-EBP-β *via* inhibition of ERK1/2 activity. This study provides positive feedback that AntH may be one of the active components in *A. cinnamomea* with anti-cancer effects.

3.2. *Anti-inflammatory effects of antcins*

Inflammation is the first step of biological response to injury, which is necessary requisite to healing, however it often progresses to painful or chronically harmful diseases requiring pharmacological treatment [80]. The inflammatory response is a series of a well-coordinated dynamic mechanism consisting of specific vascular; humoral and cellular events that are characterized by the movement of fluids, plasma and inflammatory leukocytes (eosinophils, neutrophils, basophils, and macrophages) to the site of inflammation [81]. A variety of chemical mediators or signaling molecules such as histamine, serotonin, leukotrienes, inducible nitric oxide synthase, prostaglandins, oxygen-derived free radicals (O_2^-, $^{\cdot}OH$, $ONOO^-$) are produced by inflammatory and phagocytic cells predominantly in the sequences which participate in onset of inflammation [82]. Natural products have long been used as traditional medicine for treating various inflammatory diseases such as fever, pain, migraine, and arthritis [83]. There is mounting evidence in support of the efficacy of phytochemicals that are potent anti-inflammatory agents [84–87]. Likewise, several studies have indicated that *A. cinnamomea* and *A. salmonea*, and their derived compounds possessed strong anti-inflammatory properties [18,26]. An overview of the anti-inflammatory properties of antcins, and their derivatives and analogs were reviewed in the following section, and the detailed mechanisms were illustrated in Fig. 5.

Polymorphonuclear neutrophils are the major component of the nonspecific cell-mediated immune system, play the first line of defense against bacterial infections [88]. Upon stimulation of neutrophils with chemotactic factors such as N-formyl-methionyl-leucyl-phenylalanine (fMLP) or phorbol 12-myrstate-13-acetate (PMA), they trigger inflammatory responses, including generation of reactive oxygen species, phagocytosis, chemotaxis, and release of anti-microbial molecules from granules to attack invading microorganisms [89,90]. It has been observed that pretreatment with 1–25 μM of AntB (**3/4**), AntH (**11/12**), AntI (**13/14**), and AntK (**15/16**) exhibited potent inhibitory activity against fMLP and PMA-induced ROS generation in cultured human peripheral neutrophils. These compounds induce firm adhesion, without interfering the induction of surface Mac-1 (CD11b/CD18), an integrin β2-mediated firm adhesion of neutrophils to endothelium [91]. A similar study also shown that AntA (**1/2**) and AntB (**3/4**) ameliorated fMLP-induced superoxide generation in cultured human neutrophils with an IC_{50} value of 8.55 ± 1.04 and 9.82 ± 4.40 μM, respectively [92]. The fMLP inhibitory effect of antcins are better than ibuprofen, a clinically used anti-inflammatory drug exhibited IC_{50} value of 27.5 ± 3.45 μM. These reports providing an additional support for the anti-inflammatory effects of Antcins.

Anti-inflammatory ergostanes, AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), AntI (**13/14**), AntK (**15/16**), AntM (**17**),

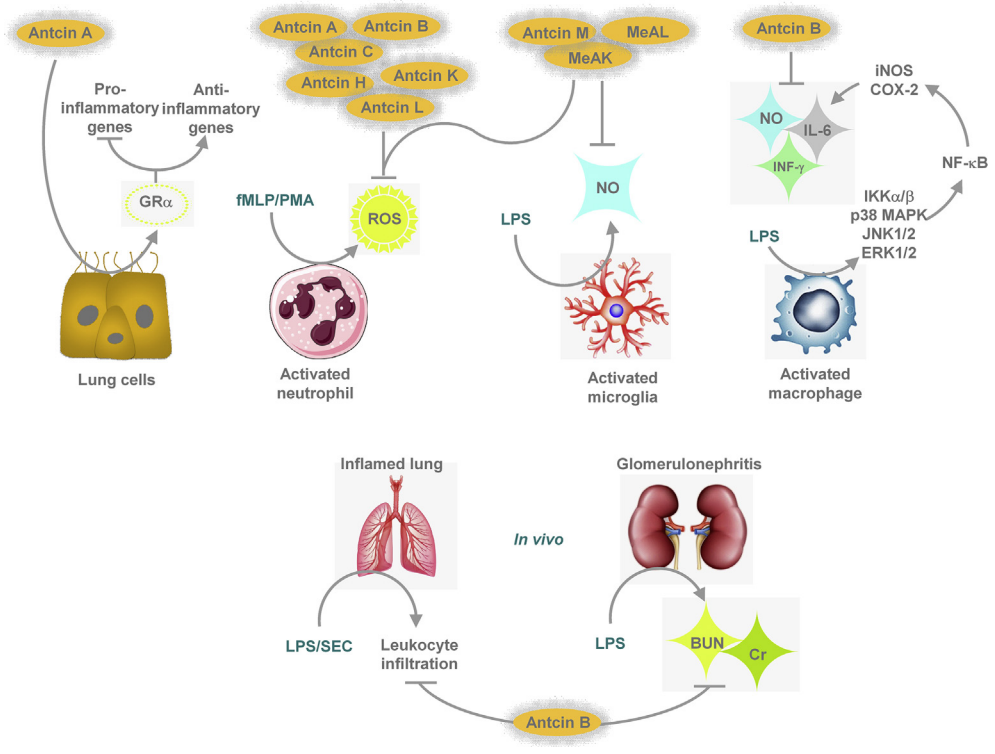


Fig. 5 – Molecular mechanisms involved in anti-inflammatory effects of antcins.

MeAK (**26/27**), and MeAL (**28**) were isolated from the fruiting bodies of *A. salmonea* [30]. The anti-inflammatory potential was determined by inhibition of reactive oxygen species (ROS) and nitric oxide (NO) production in neutrophils and microglial cells, respectively. Among them, AntM (**17**), MeAL (**28**), and, MeAK (**26/27**) displayed the potent anti-inflammatory activity in LPS-induced microglia cells as indicated by reduced levels of NO production in the culture media of activated inflammatory cells with IC_{50} values ranging from 1.7 to 16.5 μM and were more potent than a non-specific nitric oxide synthase (NOS) inhibitor. Also, these three compounds showed potent anti-oxidant activity in cell culture system with IC_{50} values around 2.0–8.8 μM, whereas no evidence of direct free-radical scavenging properties. This study conclude that AntM (**17**), MeAL (**28**), and MeAK (**26/27**) have the anti-inflammation and antioxidant properties. Chen et al. [93] isolated and purified five major Antcins (AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), and AntK(**15/16**)) from the methanolic extracts of fruiting bodies of *A. cinnamomea*; and the anti-inflammatory effects of antcins were investigated *in vitro* by determining direct glucocorticoid receptor (GR) activation. Among them, AntA (**1/2**) exhibited a strong anti-inflammatory effect when compared with other four antcins. This effect demonstrated that AntA (**1/2**) significantly induced GR activation as evidenced by increase of nuclear export in human lung cells A549. The GR agonistic effect of AntA (**1/2**) was highly comparable with the synthetic glucocorticoids such as dexamethasone and cortisone. Chemically, AntA (**1/2**) is distinguished from other isolated antcins in the presence of hydroxyl or carbonyl groups attached to C_7 of the ergostane skeleton (Fig. 2). Molecular docking study revealed that C_7 of AntA (**1/2**) exposed to a hydrophobic region in the cavity of GR exhibited higher binding affinity, whereas other four antcins expelled due to the additional hydrophobic group attached to C_7. In addition, it can assume that AntE (**8**) might have a similar GR agonist effect, because of the absence of hydroxyl or carbonyl attachment at C_7. Also, it can be speculated that other antcins may act as pro-drug with the additional hydrophilic group at C_7 removed by intestinal microflora or the enterohepatic circulation remains to be investigated.

The anti-inflammatory effect of AntB (**3/4**), one of the major compound of *A. cinnamomea*, was investigated by *in vitro*, *in vivo*, and *in silico* models [94]. Pre-treatment of AntB (0.5–10 μM) dose-dependently decreased the production of pro-inflammatory molecules, NO, IL6-, and INF-γ as well as pro-inflammatory mediators iNOS and COX-2 expression in LPS-induced murine macrophage cells without exhibiting cytotoxicity up to a concentration of 30 μM. Further luciferase reporter analysis revealed that the inhibition of iNOS and COX-2 by AntB (**3/4**) was associated with suppression of transcriptional activity of NF-κB. This effect was further explained by that AntB (**3/4**) inhibited LPS-induced phosphorylation of NF-κB and its up-stream kinases IKKα/β, ERK1/2, JNK1/2, p38 MAPK, and AKT. Besides, AntB (**3/4**) treatment inhibited LPS-induced C3H/HeJ and C3H/HeN mice or *Salmonella enterica* subsp. enterica serovar Choleraesuis (SEC)-induced C57BL/6 mice and RAW264.7 cells. *In vivo* study supported the above notion that use of AntB (**3/4**) prevents LPS-induced lung inflammation as verified by reduced level of polymorphonuclear leukocyte infiltration in lung tissues of LPS-induced C3H/HeN mice. Similarly, AntB (**3/4**) prevented LPS-induced glomerulonephritis in C3H/HeN mice, which was explained by AntB markedly reduced the LPS-induced elevation of the biomarkers (BUN and serum creatine) kevels of kidney injury. Also, AntB (**3/4**) protected C3H/HeN mice against lethality and improved survival during endotoxemia, suggesting that AntB (**3/4**) has therapeutic potential against LPS-induced sepsis and gram-negative bacterial infections in general. It has been demonstrated that small molecules interrupt TLR4 signaling by competing the binding of LPS to MD-2 has therapeutic value [95]. A new knowledge-based scoring program, HotLig and X-score were applied to predict the molecular interaction and affinity between AntB (**3/4**) and hydrophobic amino acid residues of MD-2, which constitute the LPS binding pocket. Docking results demonstrate that AntB could bind the free MD-2 with ~85%–90% successes rate, predicted by HotLig. On the other hand, X-score predicted the binding affinity (pKd) of AntB was 7.83, whereas that of LPS was 5.83. This study explained the molecular mechanism that AntB (**3/4**) able to bind TLR4/MD-2 receptor by competing with LPS.

3.3. *Immunomodulatory effects of antcins*

Recent advances in immunology manifested the understanding of host immune responses to infectious diseases. Several cell surface and soluble signaling molecules produced by immune cells have been discovered that eventually regulate host response towards microorganisms [96]. In the past three decades, scientists have focused on discovering agents that positively or negatively modulate the biological response of immune cells, thereby enhanced host ability to resist microbial infection. Several agents, including plant and microbial extracts, and their derived compounds such as peptides, polysaccharides, glycoproteins, lipids, phenolic compounds, alkaloids, flavonoids, anthocyanins, tannins, saponins, terpenoids, steroides, and lectins have all been characterized as small molecules that have potent effect on immune system [97–99]. Lu et al. [26] and Geethangili and Tzeng [18] were extensively reviewed the immunomodulatory effects of *A. cinnamomea* and their derived compounds. The following section reviewed the immunomodulatory effects of antcins and their involved molecular mechanisms.

AntK (**15/16**), AntN (**18**) and their analogs, MeAK (**26/27**) and MeAN (**29**) were isolated from the fruiting bodies of *A. cinnamomea* or *A. salmonea*. The immunomodulatory effect of antcins were investigated in bone marrow dendritic cells (BMDCs). Among them, MeAK exhibited a pronounceable activity [100]. It was found that MeAK (50 μM) promoted DCs maturation via up-regulating major histocompatibility complex (MCH) class II, CD86, and CD54, whereas MAK failed to up-regulate CD80 and CD40 in DCs. It further extended by that MeAK reduced endocytosis of a large molecules, such as dextran. Besides, MeAK reduced innate immunity as indicated by increased production of pro-inflammatory cytokines and chemokines, such as tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1β (MIP-1β) in DCs. However, interferon-γ (INF-γ), interleukin-1α (IL-1α), IL-1β, IL-2, IL-6, IL-10, IL-12, MIP-1α, and RANTES were not significantly produced by MeAK.

Furthermore, MeAK activated DCs induced OT-II and Ag-specific T-cell proliferation *in vitro* and *in vivo*, respectively; it confirming that DCs exposed to MeAK facilitated antigen-specific T-cell proliferation. Further analysis revealed that MeAK-activated DCs promoted polarization of T-cells to the Th2 phenotype, as shown by increased expression of IL-4, IL-5, IL-13, and GATA genes.

A panel of antcins, including AntA (**1/2**), AntB (**3/4**), AntB methyl ester, AntC (**5/6**), AntH (**11/12**), MeAH (**24/25**), and AntI (**13/14**) exhibited immunomodulatory activity by inhibiting the production of TNF-α, cytokines (IL-6, IL-12), and chemokine (RANTES) in LPS-stimulated BMDCs. This finding indicated that these seven compounds potentially possess the ability to inhibit the activation of DCs. Indeed, comparing the inhibitory effect, AntH exhibited the highest inhibitory activity among them. Further analysis showed that treatment of AntH dose-dependently reduced TNF-α, IL-6, IL-12 and RANTES levels in LPS-induced BMDCs. A significant inhibition was found over a concentration of 25 μg/mL AntH. Maturation of DCs is known to be essential step in the stimulation of the adaptive immune response after DCs activation [101]. Result of flow cytometry analysis revealed that the use of AntH decreased the expression levels of MHC II molecules, as well as co-stimulatory molecules CD40 and CD80 in LPS-stimulated DCs. In mice, contact hypersensitivity response has been studied using haptens such as dinitroflorobenzene (DNFB) to determine the immunosuppressive effect *in vivo* [102]. Result of histopathological analysis showed that the tested ears were swollen in DNFB-sensitized mice, whereas ear swelling was not detectable in DNFB plus AntH-sensitized mice. Also, number of infiltrative T cells ($CD3^+$) in ears of AntH + DNFB treated mice were comparatively lower than that of DNFB-sensitized group.

3.4. *Hepatoprotective effects of antcins*

Traditionally, the fruiting bodies of *A. cinnamomea* has been well-known for its hepatoprotective properties. Recent scientific studies clearly demonstrated preventive or ameliorating of liver diseases, such as preventing alcohol- and CCl_4-induced liver injury, ameliorating fatty liver and liver fibrosis, inhibiting the hepatitis B virus, and even inhibiting liver cancer growth. The hepatoprotective efficacy of *A. cinnamomea* and its derived compounds were extensively reviewed [23,25,27]. Fig. 6 summarizes the hepatoprotective mechanisms of antcins based on the published results. 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), a free radical generating azo compound-induced oxidative stress in liver cells in an experimental model [103], we examined the hepatoprotective effects of AntC (**5/6**) on hepatocellular carcinoma HepG2 cells [104]. We found that pre-treatment of AntC protects hepatic cells from AAPH-induced cell death via suppressing excessive production of reactive oxygen species (ROS). Additionally, pre-treatment with AntC limited the APPH-induced secretion of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and glutathione (GSH) depletion. Furthermore, the preventive effect of AntC was achieved by induction of endogenous anti-oxidant genes, including hemoxygenase-1 (HO-1), NAD(P)H Quinone dehydrogenase-1 (NQO-1), γ-glutamylcysteine (γ-GCLC) and superoxide dismutase (SOD) through the redox-sensing transcription factor nuclear factor erythroid 2-related factor-2 (Nrf2). On other hand, AntC prevents AAPH-induced apoptosis in HepG2 cells through the down-regulation of pro-apoptotic factors, such as Bax, cytochrome c, capase 9, −4, −12, −3, and PARP. The effect of AntC on hepatoprotection *in vivo* was examined, showing that pre-treatment with AntC protected mice liver from AAPH-induced hepatic injury as evidenced by reduction of hepatic enzymes in circulation and elevated levels of GSH in hepatic tissues. Immunocytochemistry analyses showed that AntC significantly up-regulated HO-1 and Nrf2 expression in AAPH-administered mice liver tissues. This study conclude that AntC could protect liver cells from oxidative stress and cell death via Nrf2/ARE activation. Another study have shown that AntH (**11/12**) prevent and treat acetaminophen (APAP), galactosamine (GalN)/TNF-α-induced hepatic injury in mice, as indicated by decreased levels of ALT in the mice serum [105]. In agreement with the changes of ALT, histopathological analysis revealed that AntH significantly attenuated APAP or GalN/TNF-α-induced severe zone III necrosis of hepatic lobules. Mitochondrial translocation of c-Jun-N-terminal kinase (JNK) followed by phosphorylation is an exacerbating event in APAP-induced hepatotoxicity [106]. Treatment with AntH (**11/12**) prevented sustained JNK phosphorylation and mitochondria translocation, thereby halted excessive ROS production both APAP-induced mice liver tissue and GalN/TNF-α-induced primary hepatocytes. A recent study have shown that AntK (**15/16**) prevents *N*-nitrosodiethylamine-induced liver inflammation in mice [107]. Treatment with Ant-K significantly reduced N-nitrosodiethylamine-induced hepatic ROS, bile O_2^-, H_2O_2, and γ-GT levels *via* suppressing the NF-κB transcriptional activity in mice. The reduced NF-κB activity by AntK was reasoned by inhibition of its up-stream kinases including p85/phosphoinositide-3-kinase (PI3K), mitogen activated protein kinase (MAPK), and CYP2E1 expression. In addition, immunohistochemistry and TUNEL assays revealed that AntK significantly inhibited *N*-nitrosodiethylamine-induced autophagy and apoptotic cell death in hepatic tissue as indicated by reduced levels of baclin-1 expression and TUNEL-positive cells.

Li et al. [108] investigated the hepatoprotective effect of various samples/culture conditions of *A. cinnamomea*, including wild growing fruiting bodies, cutting wood culture fruiting bodies, dish cultures, cutting wood culture mycelia, and submerged fermentation mycelia in carbon tetrachloride (CCl_4)-induced hepatic injury in mice. Result showed that the wild growing, cutting wood culture fruiting bodies, and the dish cultures of *A. cinnamomea* showed more potent activities than the mycelia. The results imply that cultivation techniques remarkably affect the hepatoprotective activities of *A. cinnamomea*. Besides, ten antcins and their derivatives, including 25(*S*) AntB (**3/4**), 25(*R*) AntB (**3/4**), 25(*S*) AntC (**5**), 25(*R*) AntC (**6**), AntF (**9**), 25(*S*) AntH, 25(*R*) AntH, 25(*S*) AntI (**13**) 25(*S*) AntK (**15**), and 25(*R*) AntK (**16**) were isolated from various samples of *A. cinnamomea* and exhibited significant protective effect as indicated by increased of HepG2 cell survival from 46% to >90% in CCl_4-challenged condition. Further *in vivo* analysis revealed among the tested antcins, pre-treatment of AntB (**3/4**) and AntK (10 or 50 mg/kg for 7 days) dose-dependently decreased CCl_4-induced hepatic injury as

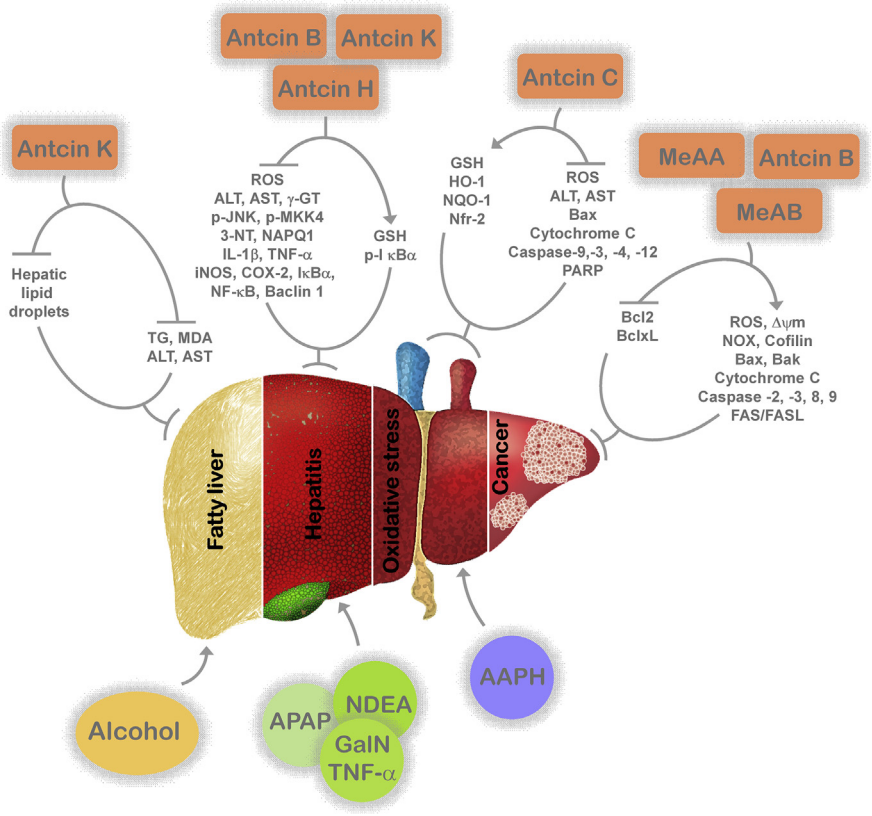


Fig. 6 – Molecular mechanisms involved in hepatoprotective effects of antcins.

evidenced by decreased level of serum ALT and AST, and hepatic necrosis. The preventive effect of AntB (**3/4**) and AntK at 50 mg/kg were almost identical to those of silymarin 100 mg/kg treatment group. Further molecular analysis revealed that use of AntB (**3/4**) and AntK (**15/16**) significantly down-regulated IL-1β, TNF-α, iNOS, COX-2, and NF-κB in hepatic tissues at both transcriptional and translational levels.

Three types of liver diseases related to alcohol consumption, are fatty liver, alcohol hepatitis and cirrhosis. Alcoholic fatty liver is a common and universal syndrome among the chronic heavy drinkers and above 40% those with moderate alcohol consumption (1–80 mg/day) also exhibit fatty liver formation and generally reversible with abstinence [109]. Although preventing alcohol consumption, the management of alcoholic fatty liver diseases only involves preventing excessive lipid accumulation [110]. The original use of wild *A. cinnamomea* by indigenous people of Taiwan is to overcome from the alcohol intoxication. Mycelia and sporocarp extracts of *A. cinnamomea* prevents alcohol-induced fatty liver and hepatitis in rats [111,112]. A recent study reported that the methanol extract of petri dish cultured *A. cinnamomea* and its main compound, AntK (**15/16**), showed hepatoprotective effect against alcohol-induced liver injury in mice [113]. Treatment of AntK 10, 30, and 60 mg/kg/day for 30 days prevents alcohol-induced alteration in serum biochemistry and fatty liver formation. Over a dose of 30 mg/kg AntK significantly reduced the plasma levels of TG, MDA, and AST in alcohol-induced mice; whereas all the tested concentrations of AntK significantly reduced the plasma level of ALT. Histopathological analysis further support this notion that AntK pretreatment markedly blocked alcohol-induced lipid droplet formation in hepatocytes in a dose-dependent manner. This study indicated that pre-treatment of AntK could prevent alcohol-induced fatty liver formation.

3.5. *Anti-diabetic and hypolipidemia effects of antcins*

Diabetes mellitus (DM) is a chronic metabolic disorder that characterized by insulin resistant and hyperglycemia. DM was pathologically classified into two types, type-I and type-II. In type-I, the pancreas unable to secrete insulin due to autoimmune destruction of the insulin producing β-cells. Type-II DM, the pancreatic β-cells can produce insulin, whereas the endothelial or muscle cells cannot use it efficiently, a condition known as insulin resistance [114]. When the cells insensitivity to insulin, the blood sugar (glucose) gradually increased and triggered several metabolic and cardiovascular diseases [115]. Many plant extracts and their derived compounds have been reported to show antidiabetic activity [116]. AntK (**15/16**), a major steroid of the fruiting bodies of *A. cinnamomea* displayed anti-diabetic and hypolipidemia effects on high fat diet (HFD)-induced mice model [117]. Skeletal muscles are the largest tissue in our body and account for the majority (~75%) of insulin-mediated glucose uptake in the post-prandial state and regulating blood glucose homeostasis. Recently, several phytochemicals has been reported to promote glucose uptake by activation of PI3K/AKT or AMPK-mediated plasma translocation of glucose transporter-4

(GLUT4) in skeletal muscle cells [118]. An initial *in vitro* analysis have shown that treatment of C_2C_{12} myoblast cells with AntK (1–25 μg/mL) significantly as well as dose-dependently increased expression of GLUT4 and phosphorylation of AKT at Ser^{473} and AMPK at Thr^{172} residues. The effect of AntK (**15/16**) was highly comparable with insulin treatment. Next, treatment with AntK (10–40 mg/kg/day) significantly lowered the HFD-induced elevation of blood glucose, triglyceride, total cholesterol, and leptin and increased adiponectin levels in a dose-dependent manner. Of note, the anti-hyperglycemic effect of AntK at a dosage of 40 mg/kg/day is comparable to Metformin (300 mg/kg/day) and the anti-hypertriglyceridemic effect was highly comparable to Fenofibrate (250 mg/kg/day). In addition, AntK (**15/16**) inhibited HFD-induced hepatic fatty acid synthase (FAS) in hepatic and adipose tissues, whereas increase of fatty acid oxidation peroxisome proliferator-activated receptor-γ (PPAR-γ) expression coincident with decreased level of sterol-response element binding protein-1c (SREB-1c) in hepatic tissue may contributed to decreased levels of plasma triglycerides, total cholesterol, and hepatic steatosis. This study is consistent with a previous report that eburicocic acid, a lanostane type steroids isolated from *A. cinnamomea* reduced triglyceride and total cholesterol levels in streptozotocin-induced diabetic mice [119]. Another study also support the anti-diabetic properties of antcins that treatment with AntK significantly increased GLUT4 expression through the activation of AKT by phosphorylation at Ser^{473} residue in C2C12 murine myoblast cells [120].

Peroxisome proliferator-activated receptor α (PPARα), a member of ligand-activated transcription factors of nuclear hormone superfamily that transcriptionally regulates lipid metabolism and energy homeostasis [121]. PPARα agonists such as fibrate drugs (clofibrate, gemfibrozil, ciprofibrate, bezafibrate, and fenofibrate) have been used for the treatment of hyperlipidemia and metabolic diseases due to their ability to lower plasma triglyceride level and elevate high-density lipid cholesterol levels [122]. Naturally occurring dietary compounds, including nutrients and phytochemicals have been identified as PPARα ligands or modulators [123,124]. Since, the extracts of *A. cinnamomea* exhibited anti-hyperlipidemia effects *in vivo* [125]. A recent study demonstrated that steroid-like compounds AntB (**3/4**), AntH (**11/12**), and AntK (**15/16**), from *A. cinnamomea* activate PPARα, but not ergostatrien-3β-ol in cell-based transactivation assay [126]. Further *in silico* molecular docking studies revealed that antcins had significant molecular interactions with Tyr314 and His440 residues of PPARα, and these interactions were required for helix 12 stabilization. The agonistic effects of antcins may contribute for the development of safe and selective PPARα modulators. Another *in silico* modeling study have shown that Antcins are potent antagonists of Na^+/K^+-ATPase [127]. Five major antcins, AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), and AntK (**15/16**) were subjected to molecular docking with Na^+/K^+-ATPase receptor protein. Among them, Ant A exhibited higher inhibitory potency, whereas cortisone, a steroid analog with strong anti-inflammatory effects showed no detectable inhibitory effects. The antagonistic property of AntA (**1/2**) is attributed to its steroidal hydrophobic interaction with the binding pocket and the formation of three hydrogen bonds between its carboxyl group and two cationic residues around the cavity entrance of Na^+/K^+-ATPase. This study proposed that the bi-functional compound AntA (**1/2**) reduce inflammation and that enhance blood circulation via two different molecular mechanisms.

Alpha-glucosidase, a glycoside hydrolase enzyme located in the brush border of the small intestine decomposes complex carbohydrates such as starch and disaccharides into glucose, and increase blood glucose level, and triggered type II diabetes. Therefore, suppressing the α-glucosidase activity is a major therapeutic strategy of diabetes mellitus [128]. Several dietary phytochemicals have identified as potential α-glucosidase inhibitors [129]. A recent study reported that methanolic extracts of *A. cinnamomea* fruiting bodies possessed potent α-glucosidase inhibitory activity with high pH stability (pH 2–11) and thermostable properties at 40-50 °C. Then, eight major constituents were isolated from the extracts and identified as 25(*S*) AntK (**15**), 25(*R*) AntK (**16**), 25(*S*) AntI (**13**), 25(*S*) AntB (**3**), 25(*R*) AntB (**4**), eburicoic acid, dehydroeburicoic acid, and dehydrosuphurenic acid. Among them, 25(*R*) AntK and 25(*S*) AntB demonstrated greater α-glucosidase activity with EC_{50} of 0.054 and 0.21 mg/mL, respectively; whereas acarbose, an inhibitor of α-glucosidase exhibited EC_{50} of 0.278 mg/mL [130]. Notably, this is the first study demonstrated the α-glucosidase activity of antcins.

3.6. *Anti-aging properties of antcins*

Aging can be defined as the natural and inevitable biological processes. All the living organisms are multicomponent/organ system, recent studies revealed that aging affects each organ very differently, leaving some regularly renewed and some are rarely refreshed. For example, skin and intestine are constantly in flux, whereas heart and brain are renewed rarely [131]. Skin aging is induced by both intrinsic and extrinsic factors. Intrinsic aging is genetically determined degenerative aging processes that result in thin, dry, fine wrinkles, and gradual cutaneous atrophy; while extrinsic is engendered by external stimuli, such as air pollution, smoking, poor nutrition, and UV radiation [132]. Recent studies have manifested the diabetic-associated pre-mature skin aging [133,134]. We examined the anti-aging properties of Antcins, including AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), AntK (**15/16**), and AntM on high glucose (HG)-challenged human dermal fibroblasts *in vitro* and *C. elegans* nematode model *in vivo* [135]. Initial cytotoxic analysis showed that AntB (**3/4**) and AntK (**15/16**) are cytotoxic against the human dermal fibroblast (CCD966SK) cells with an IC_{50} value of 7.11 and 2.89 μM, respectively. The remaining non-cytotoxic antcins, AntA (**1/2**), AntH (**11/12**), and AntM are subjected to senescence-associated β-galactosidase (SA-β-Gal) assay to measure their ability to inhibit HG-induced premature senescence in dermal fibroblasts and found that AntM is more potent than that of AntA (**1/2**) and AntH (**11/12**). Further analysis with AntM exhibited the ability to inhibit premature senescence and growth arrest in HG-challenged cells. In depth molecular analysis revealed that AntM inhibits HG-induced cell-cycle arrest by provoking the expression levels of cell cycle regulatory proteins such as cyclin D, cyclin E, CDK4, CDK6, CDK2, and protein retinoblastoma. The HG-induced pre-mature

senescence was blocked by AntM through down-regulation of $p16^{INK4A}$, $p21^{CIP1}$, and deacetylation of p53, and FOXO-1. In addition, AntM eliminated HG-induced reactive oxygen species (ROS) production *via* activation of endogenous anti-oxidant mechanism through the induction of anti-oxidant genes, HO-1, NQO-1 *via* transcriptional activation of Nrf-2. A similar effect was also observed in human endothelial cells (HUVECs). Further *in vivo* studies shows that the life-span of *C. elegans* was dramatically increased in response to AntM treatment and protects the warms from HG-induced oxidative stress insult. This study suggest that AntM could be preventing agent for age-related disorders. A schematic representation of anti-aging mechanism of AntM is plotted in Fig. 7.

4. Advances in production and separation of antcins

We reported that the metabolite profile of *A. cinnamomea* fruiting bodies has great variation in different harvesting age and cultured in different wood substrates [136]. The chemical fingerprint was greatly varying in different strains of *A. cinnamomea* (AC-3, AC-5, AC-7 and AC-9) cultivated for nine months on *C. kanehirae* wood. In the case of antcins, high amount of AntA (**1/2**), AntC (**5/6**) and AntH (**11/12**) were observed in AC-9 strains, and AntB (**3/4**) and AntK (**15/16**) content was higher in AC-7 strain. Culture substrate also greatly influence the chemical profile of antcins in *A. cinnamomea* fruiting bodies as indicated by AntK and AntH were abundant in 9 and 12 months cultured fruiting bodies of AC-9 strain. AntB (**3/4**) and AntC (**5/6**) were greater in 6, 9, and 12 months ages samples. A significant amount of AntA (**1/2**) was observed in 9 months cultured samples. Wood substrates for solid-phase culture of fruiting bodies also determined antcins contentes as evidenced by large quantity of AntC, AntH, and AntK were obtained from *C. kanehirae* wood substrate culture, which is the original host species of *A. cinnamomea*. Indeed, a remarkable amount of AntC (**5/6**), AntH (**11/12**) and AntK (**15/16**) were observed in *C. konishii*, and AntA (**1/2**) and AntB (**3/4**) were in *Cinnamomum camphora* wood cultured samples. Later, we compared the metabolic profile of *A. cinnamomea* and its related species *A. salmonea* [61]. The five major antcins, AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), and AntK (**15/16**) and two lanostane-type steroids, dehydrosulphurenic acid, and dehydroeburicoic acid were assigned as index compounds to characterize the chemical profile of *A. cinnamomea* and *A. salmonea*. The HPLC metabolite profile showed that AntA (**1/2**) and AntC (**5/6**) content in *A. salmonea* was greater than that of *A. cinnamomea*, whereas AntB (**3/4**) and AntH were higher in *A. cinnamomea*. AntK (**15/16**) was almost similar in both *A. cinnamomea* and *A. salmonea* fruiting bodies. Although, the selected antcins were detected in both species, two more peaks were appeared only in *A. salmonea* at a retention time of 60–62 min. The ^{1}HNMR, and ^{13}CNMR analysis revealed that the two peaks were AntM (**17**) and MeAK (**26/27**) and suggested that AntM (**17**) and MeAK (**26/27**) might be important constituents for distinguishing *A. cinnamomea* and *A. salmonea* fruiting bodies.

Chen et al. [137] examined whether the phenologic behavior of *A. cinnamomea* influence their chemical composition, regular orange-red strains, and naturally occurring and blue-light-induced white strains were subjected to TLC and

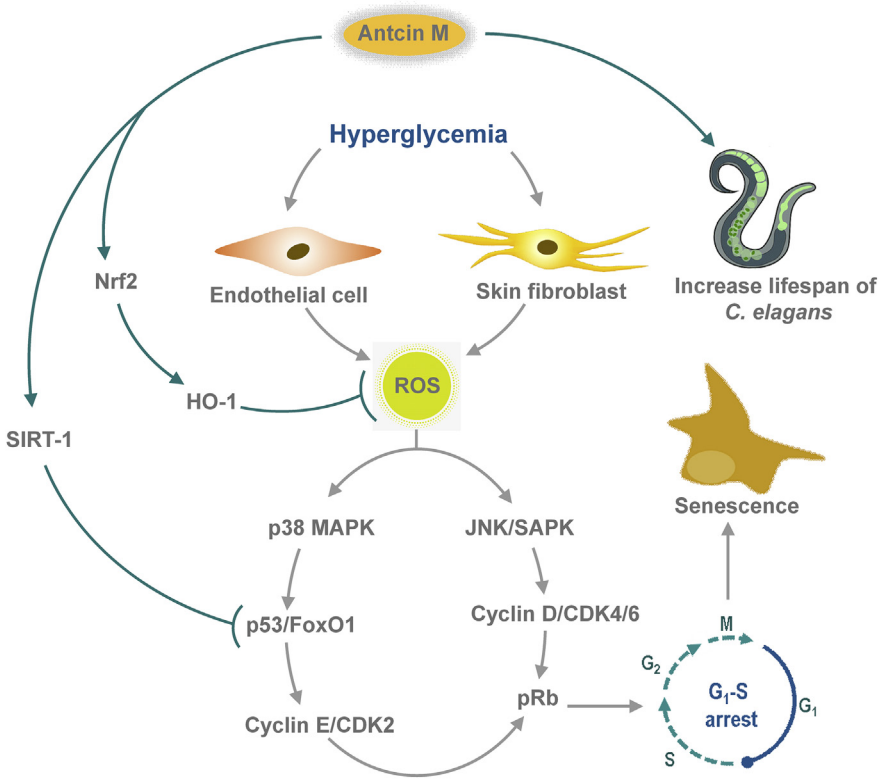


Fig. 7 – Molecular mechanisms involved in anti-aging properties of antcins.

HPLC analysis. Ten major index steroids, including six antcins, AntA (**1/2**), AntK (**15/16**), AntC (**5/6**), AntB (**3/4**), AntI (**13/14**), and AntH (**11/12**), as well as four lanostane-type steroids, dehydrosulphurenic acid, eburicoic acid, sulphurenic acid, and dehydroeburicoic acid were identified in all phenotypic strains. However, the percentage of each compound is greatly varying in all the strains. Notably, blue-light radiation (100 lm radiation of 470 nm LED light source) whitened the regular, orange-red *A. cinnamomea* and modified its growth and secondary metabolites. For example, AntK (**15/16**) was predominant in blue-light induced strains (10.987%), when compared with regular orange-red (6.808%) and naturally occurring white strain (1.105%). In addition, naturally occurring white variant did not show a better composition of triterpenoid profiles up to eight weeks old when compared with the triterpenoid profiles of the regular strain at the same age. This study also demonstrated that antcins were found in both young and aged mycelia with fruiting bodies, which was cultured in artificial agar-plate medium, suggesting that artificial agar-plate medium may yield more diversified metabolites rather than wild or submerged cultures. A recent study also support this finding that petri-dish culture of *A. cinnamomea* fruiting bodies (PDCA) yielded nineteen steroids, including four antcins with their derivatives, AntB (**3/4**), 25(*S*) AntC (**5**), 25(*R*) AntC (**6**), AntG (**10**), 25(*S*) AntK (**15**), 25(*R*) AntK (**16**), 25(*S*) MeAK (**26**), and 25(*R*) MeAK (**27**). Among them, 25(*S*) AntK (**15**), and 25(*R*) AntK (**16**), a pair of epimers were the main content of PDCA, suggesting that PDCA produced a large array of the same antcins as wild one [113].

A recent chemical finger print analysis of various culture conditions of *A. cinnamomea*, including wild grown fruiting bodies, cutting wood culture of fruiting bodies, cutting wood culture of mycelia, dish culture of mycelia, and submerged fermentation culture of mycelia found a remarkable difference in chemical composition. Preparative HPLC analysis revealed that fruiting bodies of wild grown and cutting wood culture samples were enriched with ergostane-type steroids, including AntB (**3/4**) and AntH (**11/12**) with their derivative forms, and AntK (**15/16**) was abundant in dish cultured mycelia. On the other hand, cutting wood culture mycelia contains large amount of lanostane-type steroids, such as dehydroeburicoic acid and dehydrosulphurenic acid. Whereas, either lanostane or ergostane type steroids were not found in the submerged culture mycelia samples [108]. Further chemical quality evaluation of the fruiting bodies of different *A. cinnamomea* strains and different culture conditions using phytomics similarity index demonstrated that basidiomatal portion of *A. cinnamomea* is enriched with steroids [138]. Various strains of *A. cinnamomea* fruiting bodies, BCRC-35398, AC-R02, AC-R06, and AC-W01 and different culture condition of CRC-35398 strain grown on *C. kanehirae*, *C. camphora*, and *Cunninghamia lanceolata* wood substrate for 18 months, and *A. cinnamomea* cultured in malt extract agar medium containing petri dish for 3 months. HPLC finger print from ethanolic extract of various strains and culture conditions revealed that eight index compounds, including five antcins, AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), and AntK (**15/16**) were found in all four strains and culture conditions. This study concluded that phytomics similarity index analysis could be an advanced tool for selecting bioactive compounds enriched strain or culture as well as proper clinical use and pharmacological investigation of *A. cinnamomea*.

Shih et al. [139] studied whether carbon and nitrogen sources, oxygen supply, and addition of plant oils may improve the production of biomass and bioactive metabolites, including steroids in submerged culture of *A. cinnamomea*. Results showed that addition of 3% corn steep powder, as a source of nitrogen significantly increased steroids production. In parallel, supplementation of 2% glucose, as a carbon source improved production of steroids. In contrast, supplementation of vegetable oils, such as soy bean oil, olive oil, coconut oil, peanut oil, and sunflower oil and high oxygen (30%) supply increased biomass production, whereas steroids production were inhibited. *C. kanehirae* is the only host species of *A. cinnamomea* and it has been believed that wood components of *C. kanehirae* might be associated with the bioactive components of *A. cinnamomea*. Chen et al. [140] isolated 23 volatile compounds from the wood of *C. kanehirae*, among them α-terpineol, 1-terpineol, and 4-terpineol were identified as the major volatile compounds. Likewise, 19 and 24 major volatile compounds were found in fruiting bodies and liquid cultured broth of *A. cinnamomea*, respectively. Indeed, the contents of α-terpineol, 1-terpineol, and 4-terpineol were only found in fruiting bodies of *A. cinnamomea*, whereas it couldn't be found in liquid culture broth suggesting that terpineols may involve in growth and development of basidiome. The fruiting bodies of *A. cinnamomea* only grow in the inner cavity of *C. kanehirae*, it is suggested that α-terpineol may act as an elicitor to stimulate steroids production in *A. cinnamomea*. Since, *C. kanehirae* is an endemic and endangered broadleaf wood species in Taiwan, also resources of this wood is limited in natural forest; therefore, it is difficult to cultivate *A. cinnamomea* using *C. kanehirae* wood as substrate [141]. It was reported that water soluble extract of the host related species *C. camphora* promoted the hyphal growth of *A. cinnamomea* [142]. Similarly, petroleum ether, ethyl acetate, methanol, and hot water extracts of leaves and stems of *C. camphora* induced mycelial growth of *A. cinnamomea*. Besides, petroleum ether and ethyl acetate extracts of stem and leaves promoted steroids production in submerged culture of *A. cinnamomea*. Further studies revealed that addition of α-terpineol (5 mg/L) in liquid culture significantly increased ergostane-type steroids, including antcins (AntE (**8**), AntH (**11/12**), and AntK (**15/16**)) production in submerged culture of *A. cinnamomea*, whereas the lanostane type compound sulfurenic acid was unaffected [143]. This study further proved that α-terpineol can act as a signaling molecule for antcin biosynthesis, and also associated with the basdiomatal formation of *A. cinnamomea*. A similar study demonstrated that supplementation of limonene, a monoterpenoid to the shake-flask culture of *A. cinnamomea* mycelia promoted steroids production without altering their biomass [144]. In contrast, γ-terpinene significantly ($P < 0.05$) reduced steroids production, whereas β-pinene unaffected either biomass or triterpenoid production in shake-flask culture. Next, they optimized that 1% of limonene supplementation is more effective in steroids and polyphenol production. A recent study also demonstrated that addition of monoterpenoids, tangerine oil, and exposed to red light increased production of biomass and steroids in

submerged culture of *A. cinnamomea* [145]. It was observed that addition of monoterpenoids, limonene, β-pinene and terpionolene significantly increased steroids production, whereas mycelial biomass was significantly decreased with increasing concentrations of all monoterpenoids. The optimized concentration of 3 mL/L, 1 mL/L, 3 mL/L of limonene, terpionolene, and β-pinene resulted highest terpenoids production. In addition, supplementation of limonene (3 mL/L) and terpionolene (1 mL/100 mL) in the presence of red light (300 lx) further increased steroids production and limited inhibition of mycelial growth. This result was somewhat contrast to a previous report [144] that terpionolene and β-pinene did not enhanced steroids production in liquid culture of *A. cinnamomea*. It could be concluded that difference of media components, concentration, addition time, and irradiation with red light could be the reason for dissimilar results pattern [145].

Another study found that supplementation of citrus fruit peel powder enhanced the production of bioactive steroids in petri-dish solid-state fermentation culture [146]. It is interesting to note that supplementation of 2 g/plate of citrus fruit, including lemon, orange, tangerine, and grapefruit peel powder increased the contents of crude steroids. Particularly, grapefruit peel powder exhibited pronounceable effect as indicated by increased of crude triterpenoids from 9.66 mg/g DW to 36.16 mg/g, which had more than 3-fold increase of control sample. Next, a comparative HPLC analysis with wild basidiomes and mycelia extracts from petri-dish cultures with or without grapefruit peel powder revealed that five antcins, *i.e.* AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), AntI (**13/14**), and AntK (**15/16**) were found in wild fruiting bodies, whereas AntB (**3/4**), AntH (**11/12**), and AntI (**13/14**) were found in mycelia extract without grapefruit peel powder. Indeed, all five addition of grapefruit peel powder (4 g/plate) appeared all five antcins in the mycelial extract after 30 days fermentation. This study highlighted the useful of citrus peel powder for the efficient production of biomass and bioactive metabolites in solid-state fermentation of *A. cinnamomea*.

In addition to synthetic or natural resources, biotransformation is an alternative e route for developing new drug candidates. Xenobiotics can be bio-transformed into new compounds or analogs via microorganisms or enzymes. A recent study shows that *Bacillus subtilis* ATCC 6633 biotransform ganoderic acid, a major lanostane-type triterpenoid in *G. lucidum* into ganoderic acid −15-O-β-glucoside by uridine diphosphate-dependent glycosyltransferaces, whereas glycosylation of AntK (**15/16**), a major ergostane-type steroid from the fruiting bodies of *A. cinnamomea* was not affected by glycosyltransferaces from *B. subtils*. It was further revealed that AntK contains C_3, C_4, and C_7 hydroxyl and C_{26} carboxyl group but lacks the C_{15} hydroxyl group required for glycosylation of AntK or ergostane-type steroids [147]. Despite the fact, a recent study demonstrated that AntK derivatives, 25(*S*)AntK (**15**), and 25(*R*)AntK (**16**) were bio-transformed into antcamphin E and antcamphin F by soil-isolated bacterium *Psychrobacillus* sp. AK1817, catalyzed dehydrogenation of the C_3 hydroxyl group on the AntK. However, *B. megaterium* ATCC 14581 failed to produce AntK metabolites in a similar condition [148].

Male et al. [149] reported that antcins, AntB (**3/4**), AntH (**11/12**), and MeAB (**21/22**) isolated from the fruiting bodies of *A. cinnamomea* showed inhibitory effect against *Spodoptera fruqiperda* Sf9 insect cell. Particularly, MeAB exhibited a pronounceable inhibitory effect. This study also demonstrated that replacing their ketone group with hydrogen or hydroxyl group or addition of methyl or carboxylmethyl groups significantly reduced their inhibitory effects.

Ergostane-type steroids, antcins and antachamphins were identified as major bioactive constituents of the medicinal mushroom *A. cinnamomea*. These tetracyclic steroids usually occur as 25*R*/*S* epimeric pairs, which renders their chromatographic separation difficult. To overcome this hurdle, Qiao et al. [150] optimized the conditions of supercritical-fluid chromatography (SFC), including stationary phase, mobile phase, and instrumental parameters. The (*R*) and (*S*) forms for AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), and AntK (**15/16**) were well resolved ($R_s > 1.3$) on a Chiralcel OJ-H column (4.6 × 250 mm, 5 m, chiral), eluted by 10% MeOH in CO_2 at 2 mL/min with a back pressure of 120 bar and a column temperature of 40 °C. In addition, two chiral columns, Chiralpak IC (3,5-dichlorophenylcarbamate cellulose) and Chiralcel OJ-H (4-methylbenzoate cellulose), together with three achiral columns, *viz* Princeton 2-ethylpyridine, Princeton SFC Diol, and Agilent Zorbax RX-SIL, rapidly and efficiently separate low-polarity epimers like AntA (**1/2**) and AntB (**3/4**), which were very difficult for RP-HPLC. It was found that OJ-H column suffered from peak overlapping of different pairs of antcins. However, Princeton 2-ethylpyridine column successfully separated different pairs of antcins, whereas the resolutions of 25*R*/*S* forms of each epimers were not compromised with OJ-H column. These results demonstrated the different selectivity of chiral- and achiral-SFC in separating 25*R*/*S*-antcin epimers. In addition to high separation efficiency, SFC also showed advantage over HPLC in short analysis time and low consumption of organic solvents [61].

A recent study by Qiao et al. [151] elucidated the metabolism and pharmacokinetics of antcins in experimental animal model. Ethanol extract of fruiting bodies of *A. cinnamomea* (1.5 g/kg) was orally administered to rats and blood samples were collected from arteria cervicalis at 0.5, 1.5, and 12 h after administration. Eighteen steroids, including AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), AntI (**13/14**), and AntK (**15/16**) with their derivatives were found in rat plasma. On the other hand, rats were administration with 50 mg/kg of AntC (**5/6**), AntH (**11/12**) and AntK (**15/16**) and after 1.5 h blood samples were collected and antcin metabolites were determined. HRMS analysis showed that AntB (**3/4**) undertook hydrogenation (C_3 or C_7 carbonyl groups) or hydroxylation to produce polar metabolites (AntB + 4H + OH, AntB + 2H + OH, AntB + OH, and AntB + 2H). Likewise, AntH + OH was characterized as monohydroxylated metabolite of AntH (**11/12**). AntA (**1/2**) was metabolized into 3-hydroxy antcin A. However, the high polarity antcin, AntK (**15/16**) was metabolically stable. Further multi-component pharmacokinetic analysis showed that ergostaen-type steroids absorbed and eliminated rapidly, whereas lanostane-type compounds remained in the plasma at a low concentration for a relatively long time. For example, AntA (**1/2**) showed fairly low concentration and could be detected in plasma samples in certain time point,

whereas AntB (**3/4**) was below the lowest dynamic rage. These results suggest that high-polarity antcins are the major plasma-exposed components of *A. cinnamomea*, and may play an important role on its therapeutic value. In the same year, Wang et al. [152] examined the intestinal absorption of antcins using human colonic epithelial cell monolayer model. The bidirectional transport of ergostane and lanostane-type steroids through the monolayer at different time points was monitored by a fully validated LC/MS/MS system. *Trans*-epithelial electrical resistance (TEER) assay showed that the ergostane-type steroids, AntB (**3/4**) and AntH (**11/12**) were readily pass through the colonocytes as indicated by P_{AB} value (the apparent permeability coefficients from apical to basolateral side) was $>1 \times 10^{-5}$ cm/s, whereas lanostane-type steroids, dehydroeburicoic acid, and erburicoic acid were couldn't hardly pass through (P_{AB}, $< 1 \times 10^{-6}$ cm/s). In addition, the efflux ratios (P_{AB}/P_{BA}) of AntB (**3/4**) and AntH (**11/12**) were >2.0 indicating an involvement of apical efflux transporters. Indeed, AntA (**1/2**), AntB (**3/4**), AntC (**5/6**), AntH (**11/12**), and AntK (**15/16**) were absorbed by colonocyes via passive trans-cellular diffusion and exhibited high P_{AB} and P_{BA} values, whereas the lanostane-type steroids, dehydroxysulphurenic acid, and 15α-acetyldehydrosulphurenic acid displayed poor permeability. This study suggest that ergostane-type, particularly high-polarity antcins, AntB (**3/4**) and AntH (**11/12**) could be absorbed by intestinal epithelial cells.

5. Conclusion and future perspectives

Overall, the present review compiled the pharmacological properties of antcins, mainly for anti-cancer, anti-inflammation, hepatopreotection, anti-diabetic, anti-aging, hypolipidemic, and immunomodulation; and advances in production were reported from 1995 to 2019, mainly done by several research groups in Taiwan and China. These *in vitro* and *in vivo* studies provided solid evidence for the potential of antcins to develop therapeutic agents for complicated diseases, such as cancer and diabetes. However, there has never been any reports about their therapeutic significance in human clinical trials. The next step should be testing toxicity and randomized controlled clinical trials with antcins. Antcins belongs to a relatively new class of triterpenoid/phytosterol whose made of biosynthesis has been neither clarified nor proposed. A series of natural analogs of antcins have been identified in recent years, which exhibit increased potency and wide-range of selective activities compared to the parental compounds. For examples, methyl antcinate A showed anti-cancer effects to a wide variety of cancer cells than that of antcin A. Since antcins mimic as steroid/glucocorticoid, future insights into the structure–activity relationship of antcins and its derivatives are expected to promote the development of this very promising class of natural compounds into therapeutic agent and their transfer into clinical application.

Declaration of Competing Interest

All authors declare no conflicts of interest.

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