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Review

Sesquiterpene lactones of *Aucklandia lappa*: Pharmacology, pharmacokinetics, toxicity, and structure–activity relationship

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ABSTRACT

The medicinal part of *Aucklandia lappa* (Asteraceae) is its dried root, which is one of the commonly used Chinese medicinal materials. Here we reviewed sesquiterpene lactones isolated from *A. lappa* over the past ten years in the following aspects of pharmacological activities, pharmacokinetics, toxicology, structure–activity relationship. Pharmacological activities consist of anti-cancer, anti-inflammatory activity, anti-immunity activity, anti-oxidant activity, antimicrobial activity, spasmolytic activity and so on. The extractive, showing similar pharmacokinetics parameters, may exert their various biological activities by the interaction of their α-methylene-γ-butyrolactone moiety with the thiol groups of biomacromolecules through Michael-addition. However, the poor aqueous solubility, non-selective binding as a Michael acceptor at undesired targets limited clinical translation of this class. In order to evaluate the potential effect of the extractive applied in clinical trial, the present review outlines information on pharmacological activities, pharmacokinetics, toxicology, and structure–activity relationship, as well as the future research directions of the extractive for further development and utilization of *A. lappa*.

Contents

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1. Introduction

Aucklandia lappa Dence (Asteraceae) introduced from India has been cultivated in Southwest China. *Aucklandiae Radix*, known as Muxiang in Chinese, is derived from the dried roots of *A. lappa*, and it is a kind of domestic health food as well as ornamental plant in China market (Abd Eldaim, Tousson, El Sayed, & Awd, 2019). Moreover, *A. lappa* is one of the most important and commonly used herbs for invigorating *Qi* circulation and eliminating pain in clinical practice of traditional Chinese medicines, which has been listed as the highest grade in *The Holy Husbandman's Classic on Roots and Herbs* (Committee of National Pharmacopoeia, 2015). Traditionally, it is believed to have beneficial effects on asthma, rheumatism, coughs, cholera, chronic skin diseases, influenza, quartan malaria, leprosy, persistent hiccups, rheumatism, stomach-ache, toothache, and typhoid fever by means of alone usage or combining with other herbs (Seo, Lim, Jeong, & Shin, 2015).

Recent years, substantial studies on *A. lappa* in various aspects have been conducted. So far, many chemical components have been isolated, including terpenoids, tannins, steroids, alkaloids, glycosides, avonoids, peptides, and organic acids. Yin, Qi, Hua, and Pei (2005) isolated eight compounds from *A. lappa* which were identified by ^{1}H NMR, ^{13}C NMR and MS as dehydrocostus lactone, costunolide, arbusculinA, betulin, 5-hydroxymethyl-2-furaldehyde, 3,5-dimethoxy-4-hydroxybenzaldehyde, *n*-butyl-β-*D*-fructopyranoside, and 1-oleoylglycerol. Thus it can be seen that sesquiterpenes are the main components of *A. lappa* and the important active components, which consists of a wide variety of sesquiterpene lactones. A portion of primary chemical structures of sesquiterpene lactones isolated from *A. lappa* ("extractive" was used as a substitute for "sesquiterpene lactones isolated from *A. lappa*" mentioned later) showed in Fig. 1 (Wei, Peng, Ma, Xu, & Xiao, 2012). Phytochemical investigations showed that sesquiterpene lactones were the most effective components of *A. lappa* and have many pharmacological activities, such as anti-cancer, anti-inflammatory, anti-immunity, antioxidant activity, antimicrobial activity, spasmolytic activity and so on. Especially, costunolide and dehydrocostus lactone, two main bioactive compounds of the extractive, have been studied most because of their excellent pharmacological activities both *in vivo* and *in vitro*. Recent studies have also concentrated the pharmacokinetics of sesquiterpene lactones isolated from *A. lappa* as well as structure–activity relationship (Pandey, Rastogi, & Rawat, 2007).

The publications retrieved from PubMed, ScienceDirect, Springer, and Wiley databases were collected and summarized for the last 10 years. However, several papers published before 2009 were also included for insight in the introduction and explanation. In this review, we intend to provide comprehensive insights into pharmacological activities, pharmacokinetics, toxicology, and structure–activity relationship of the extractive for further development and utilization.

2. Pharmacological activities

2.1. Anti-inflammatory and anti-immunity activities

Many researches have studied the anti-inflammatory activity of the extractive. Santamarin, a sesquiterpene lactone isolated from *A. lappa*, has an α-methylene-γ-butyrolactone moiety, increases heme oxygenase-1 expression through Nrf2 translocation and suppresses NO, PGE2, TNF-α, and IL-1β production through inhibition of NF-κB translocation in LPS-induced macrophages (Spencer et al., 2018). Santamarin has been demonstrated that it might have potent therapeutic effects and should be considered for further development of treatments for various inflammatory diseases. Alantolactone, a sesquiterpene lactone isolated from the roots of *A. lappa*, exerts its anti-inflammatory effect in LPS-stimulated RAW 264.7 cells (macrophage) by suppressing NF-κB activation and MAPKs phophorylation via down-regulation of the MyD88 signaling pathway, which suggests that it may provide a useful therapeutic approach for inflammation-associated diseases. Costunolide could attenuate LPS/*D*-galactosum-induced liver injury and might be a potential therapeutic reagent for liver injury

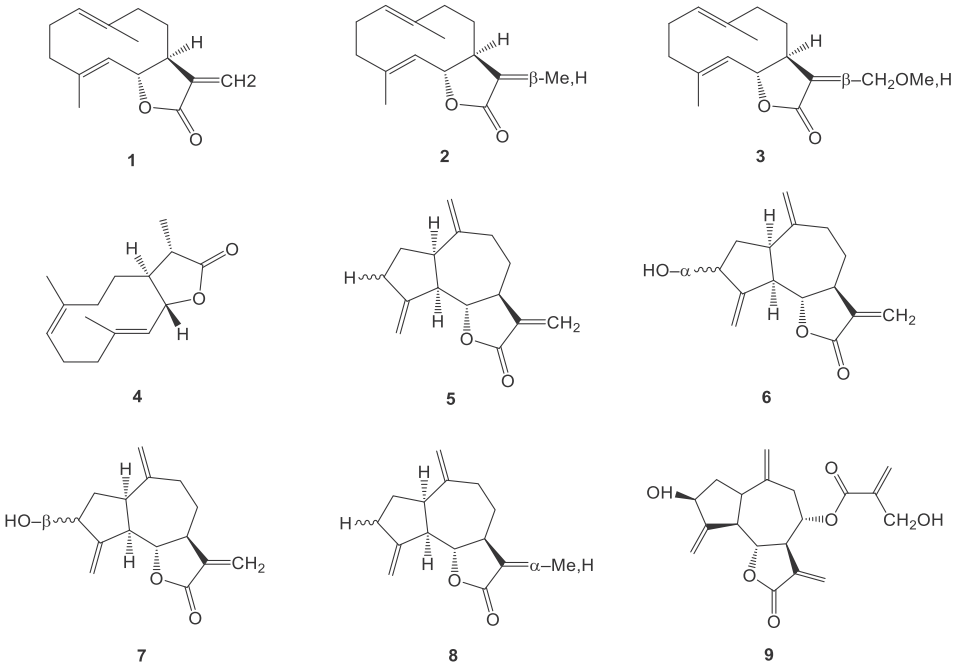

Fig. 1. Chemical structures of some main active components in sesquiterpene lactones isolated from *Aucklandia lappa*. (1 costunolide; 2 dihydrocostunolide; 3 12-methoxydihydrocostunolide; 4 dihydrocostus lactone; 5 dehydrocostus lactone; 6 α-hydroxydehydrocostus lactone; 7 β-hydroxydehydrocostus lactone; 8 mokko lactone; 9 cynaropicrin).

(Choi et al., 2012a; Rasul et al., 2013; Zhang et al., 2014). Similarly, costunolide has been demonstrated that it protects against lipoteichoic acid-induced neutrophil lung infiltration via inhibiting MAPK signaling pathway, and is a promising agent for treatment of Gram-positive bacteria-mediated pneumonia (Parekh & Chanda, 2007). Costunolide inhibited the differentiation of pro-inflammatory CD4 + T cells through the modulation of mitogen-activated protein kinases and can be an effective therapeutic agent for T cell-mediated immune diseases. A study suggested that dehydrocostus lactone may be a fundamental anti-allergic agent that can alleviate allergy, by reducing bronchial inflamation, the levels of type 2 cytokine, immunoglobulins E and mucus secretion (Pyun, Kang, Seo, & Lee, 2018). Lim et al. (2014) found that an extract of sesquiterpene lactones effectively suppressed the development of atopic dermatitis, which was closely related to the reduction in the mRNA levels and production of inflammatory chemokines and cytokine, including thymus-regulated and activation-regulated chemokine, macrophage-derived chemokine, and IL-8 in TNF-α/IFN-stimulated HaCa T cells. In the atopic dermatitis model, *A. lappa* significantly reduced the dermatitis score and levels of serum immune globulin E as well as thymus-regulated and activation-regulated chemokine (Chen et al., 2017). In addition, the back skin and ears of *A. lappa*-treated Nc/Nga mice exhibited worsen of histological manifestations of atopic skin lesions such as erosion, hyperplasia of the epidermis and dermis, and inflammatory cell infiltration (Lin et al., 2016).

Studies also showed that administration of sesquiterpene lactones decreased the number of immune cells, particularly eosinophils, and reduced the expression and secretion of Th2 cytokines (IL-4 and IL-13) in the bronchoalveolar lavage fluid and lung tissues of mice with ovalbumin-induced allergic asthma (Jeon, Kim, Kim, & Jang, 2017). Sesquiterpene lactones inhibited antigen-induced degranulation, which presented the contrast—dehydrocostus lactone > costunolide > alantolactone in terms of potency.

2.2. Anticancer activity

(Pitchai, Roy, & Banu, 2014) indicated that costunolide inhibited the cell viability of human breast cancer cells in a dose-dependent manner, leaving no significant change in the viability of the normal breast cells. The over expressed NF-κB subunits p65, p52 and p100 in the cancer cells were found to be down-regulated when treated with costunolide at an effective dose. In Silico results provided stable interactions between costunolide and the target proteins, supporting the results *in vitro*. Thus, costunolide elevates a fresh conviction for its use in breast cancer therapy for its cytotoxic efficacy and non-toxic nature (Jeong et al., 2007).

Studies have indicated that *A. lappa* and the extractive show anticancer activities by various pathways, mainly manifested in inhibition of microtubule protein, induction of cancer cell apoptosis and differentiation, inhibition of metastasis and invasion, inhibition of angiogenesis, inhibition of cell cycle progression and telomerase activity (Choodej, Pudhom, & Mitsunaga, 2018) (Table 1).

Table 1
Anti-cancer activity of extractive.

Drugs	Type of cells	Mechanisms
Costunolide	colon cancer cells	apoptosis
	chronic myeloid leukemia cells	apoptosis
	prostate cancer cells	apoptosis
	human ovarian cancer cells	apoptosis
	bladder cancer cells	cell cycle arrest
	human hepatocellular carcinoma cells	cell cycle arrest
	breast cancer cells	cell cycle arrest and apoptosis
	human ovarian cancer SK-OV-3 cells	cell cycle arrest and apoptosis
	human prostate cancer cells	cell cycle arrest and apoptosis
	lung squamous cancer cells	cell cycle arrest and apoptosis
	breast cancer cells	cell cycle arrest and apoptosis
	tumor cells	detyrosinated tubulin
	neuroblastoma cell lines	induce apoptosis Inhibition of metastasis and invasion
	breast cancer cells	inhibition of metastasis and invasion
Dehydrocostus lactone	human non-small cell lung cancer cells	apoptosis
	human soft tissue sarcoma cells	cell cycle arrest and apoptosis
	human umbilical vein endothelial cells	cell cycle arrest and suppresses angiogenesis
	human prostate cancer cells	apoptosis and Inhibition of metastasis and invasion
	human cervical cancer cells	apoptosis
Extract of *A. lappa*	breast cancer cells	cell cycle arrest and apoptosis
Volatile oil from *A. lappa* roots	prostate cancer cells	apoptosis
	breast cancer cells	cell cycle arrest and apoptosis

2.2.1. Inhibition of microtubule protein

In the context of cancer, the tubulin family of proteins is recognized as the target of the tubulin-binding chemotherapeutics, which suppress the dynamics of the mitotic spindle to cause mitotic arrest and cell death. Bocca, Gabriel, Bozzo, and Miglietta (2004) first provided evidence that costunolide interacted with microtubule protein and inhibited the growth of human breast cancer cells. Microtubules aggregated with a somewhat spindle morphology when incubation with costunolide was followed by paclitaxel, indicating that within cells these microtubule-active drugs are more effective in combination than as a single agent (Khan et al., 2018). Interestingly, the fact that costunolide rapidly induced the formation of tubulin polymers *in vitro* without exerting any stabilizing activity may suggest that costunolide may affect microtubule cytoskeleton differently from the classical microtubule-interacting agents, which stabilize or disrupt the microtubule network. It has been reported that costunolide can selectively target detyrosinated tubulin, in turn reduce the frequency of microtentacles and inhibit tumor cell reattachment independent of NF-κB activation (Kassuya et al., 2009).

2.2.2. Induction of cancer cell apoptosis and differentiation

Up to today, it is reported that apoptosis can be initiated through three pathways: the mitochondria-controlled intrinsic pathway, the membrane death receptor mediated extrinsic pathway, and the endoplasmic reticulum (ER) stress pathway (Kim, Choi, Nam, & Choi, 2018). Researches have showed extracts of *A. lappa* and monomeric compounds of the extractive significantly induced apoptosis of human prostate cancer cells, human prostate cancer cells, human non-small cell lung cancer cells, AGS gastric cancer cells, colon cancer cells, prostate cancer cells, human ovarian cancer cells, both breast and cervical human cancer cells and so on (Park, Song, Kim, Park, & Kim, 2016).

Researches reported that hexane extract of *A. lappa* and dehydrocostus lactone inhibited cell growth and induced apoptosis by increasing the levels of the pro-apoptotic proteins Bax Bak, Bok, Bik, Bmf, and t-Bid and cleaved caspase-8/9/7/3 and cleaved PARP (Choi et al., 2005; Kanno et al., 2008; Kim et al., 2008). In addition, studies have also indicated that the combination of volatile oil from *A. lappa* roots and costunolide as well as dehydrocostus lactone treatment could induce breast cancer cells differentiation and

apoptosis through c-Myc/p53 and AKT/14-3-3 signaling pathways and may be novel effective candidates for breast cancer treatment (Cheong et al., 2016). Besides, costunolide directly bound and inhibited thioredoxin reductase-1 leading to increasing accumulation of reactive oxygen species in colon cancer cells, which was involved in multiple mechanisms of apoptotic death including the activation of the ER stress pathway, alteration of Bcl2/Bax and caspases. Costunolide promoted imatinib-induced apoptosis in chronic myeloid leukemia cells via the Bcr/Abl-Stat5 pathway (Ko et al., 2005). A recent research showed that costunolide treatment inhibited the growth of ovarian carcinoma cells with an IC_{50} (half maximal inhibitory concentration) of 25 μmol/L, resulted in apoptotic cell death, increased the expression of Bax, and decreased the expression of Bcl-2 (Gismondi, Canuti, Impei, Marco, Kenzo, & Colizzi, 2013). Confocal electron microscopy showed that costunolide induced autophagy in the ovarian carcinoma cells. Western blot showed that costunolide treatment of ovarian carcinoma cells increased the expression of the LC3 II (autophagy markers), Beclin 1 (the key regulatory protein complex of autophagy), cleaved caspase-3, and cleaved caspase-9. Costunolide treatment significantly increased the levels of reactive oxygen species and reduced the ovarian carcinoma cells mitochondrial membrane potential. (Jeon, Kim, Kim, & Jang, 2017) revealed that costunolide induced osteoblast differentiation was regulated by ATF4-dependent heme oxygenase-1 expression in mesenchymal stem cells originated from embryo of mice. Therefore, costunolide can be thought to be a potential inducer of cancer cell differentiation.

2.2.3. Inhibition of cell cycle progression

The uncontrolled cell cycle undoubtedly can lead to the unlimited growth of cancer cells. The cell cycle is regulated by a complex network consisting of positive and negative cell cycle regulatory molecules, such as cyclins, cyclin-dependent kinases, Cdk inhibitors, and the activation of p53/p21/p27 pathway (Liu et al., 2011). Many researches reported that costunolide and dehydrocostus lactone could inhibit cell cycle progression mainly by increasing the G2/M phase and depleting of the G0/G1 phase in bladder cancer T24 cells, p53-mutant breast cancer cells, human ovarian cancer SK-OV-3 cells, human soft tissue sarcoma cells, human umbilical vein endothelial cells and human prostate cancer cells (Hung et al., 2010; Lee et al., 2014; Pavan Kumar et al., 2016).

It has been found that costunolide significantly induced apoptosis of human lung squamous carcinoma cells and induced cell cycle arrest at G1/S phase in a dose-dependent manner, by up-regulating the expression of p53 and Bax, and down-regulating the expression of Bcl-2 and activating caspase-3. In addition, the significant loss of mitochondrial membrane potential indicated that costunolide may induce apoptosis via the mitochondria-dependent pathway in human lung squamous carcinoma cells (Guo, Zhang, Gao, Qu, & Liu, 2014). The results above emphasized the potential effects of costunolide as an anti-cancer agent in a human lung squamous carcinoma cell line.

2.2.4. Inhibition of angiogenesis

Clinical data have affirmed that angiogenesis, a process necessary for solid tumor growth and dissemination, is a key clinical target that has potential to improve therapeutic. Studies suggested that costunolide and dehydrocostus lactone were potent angiogenesis inhibitors by blocking the VEGFR-KDR/FLK-1 angiogenic factor signaling pathway thereby being adopted as a potential novel agent in anticancer therapy (Rasul et al., 2013). Saraswati, Alhaider, & Abdelgadir (2018) reported that costunolide suppressed an inflammatory angiogenic response by attenuating the main components of the brovascular tissue, wet weight, vascularization (Hb content), macrophage recruitment (NAG activity), collagen deposition, and the levels of VEGF, IL-1B, IL-6, IL-17, TNF-α and TGF-β in a subcutaneous murine sponge model. Regulatory function of costunolide on multiple parameters of the main components of inflammatory angiogenesis has been revealed giving insight into the potential therapeutic benefit underlying the anti-angiogenic actions of costunolide (Xue, Zhang, & Chang, 2015).

2.2.5. Inhibition of metastasis and invasion

Researches have also indicated that costunolide and dehydrocostus lactone both can significantly reduce the invasion potential of cancer cells, dehydrocostus lactone can reduce migration of human prostate cancer cells by inhibiting mitochondria membrane potential-9 secretion and stimulating TIMP-2 (recombinant tissue inhibitors of metalloproteinase 2) secretion (Cai, He, & Yang, 2018). Costunolide inhibited TNF-α-induced breast cancer cells migration and invasion by decreasing p-IKK, p-IκB, and Nuclear p65 in NF-κB signalling pathways. Tabata et al found that the dehydrocostus lactone and costunolide had cytotoxic and migration/invasion-inhibitory effects against with neuroblastoma cell lines, indicating that the sesquiterpene lactones also suppressed invasion and migration of the neuroblastoma cells (Choi et al., 2012). The results above suggested that dehydrocostus lactone and costunolide are promising candidates for being developed into anticancer drugs effective against neuroblastoma.

2.2.6. Inhibition of telomerase activity

Most cancer cells have high telomerase activity than normal cells, which leads to enduring proliferation of the cancer cells and development of malignant tumors. Studies have showed that costunolide could inhibit telomerase activity by down-regulation of telomerase reverse transcriptase in human breast cancer cells and human B lymphoblastic leukemia cells (Dae & Bu, 2019). This effect was associated and mediated by a significant down-regulation of telomerase activity. Hence, costunolide could be potentially useful as a lead molecule for further experiments on the molecular mechanism of inhibition of telomerase, as well as for *in vivo* and clinical studies aimed at telomerase targeting.

2.3. Antioxidant activity

Chronic oxidative stress due to hyperglycemia may play an important role in progression of cell dysfunction in both types of diabetes, which have been demonstrated by various studies that diabetes can be inhibited by antioxidants (Lohberger et al., 2013; Peng et al., 2017; Pitchai, Roy, & Banu, 2014). A marked increase in the level of tissue thiobarbituric acid reactive substances in streptozotocin-induced diabetic rats indicated that enhanced lipid peroxidation could lead to tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals. The diabetic rats treated with costunolide and eremanthin for 60 days showed very low levels of tissue damage compared to untreated diabetic rats (Chun et al., 2012). These results showed that costunolide and eremanthin protected the various organs from tissue damage by reducing the reactive oxygen roduction.

Inducible heme oxygenase-1 acts against oxidants that are thought to play a major role in the pathogenesis of several diseases. Jeong et al suggested that α-methylene-γ-butyrolactone moiety in dehydrocostus lactone could increase cellular resistance to oxidant injury in HepG2 cells, presumably through Nrf2/ARE-dependent heme oxygenase-1 expression (Wang et al., 2017). Cheong et al. (2016) found that costunolide increased the PC12 cell viability, which was induced by hydrogen peroxide H_2O_2. Furthermore, it decreased the intracellular reactive oxygen species, stabilized the mitochondria membrane potential, and reduced apoptosis-related protein such as caspase-3. In addition, costunolide treatment attenuated the cell injury by H_2O_2 through the

inhibition of phosphorylation of p38 and the extracellular signal-regulated kinase.

It is found that dehydrocostus lactone and costunolide represent a valuable approach in the management of disease conditions of bone associated with increased oxidative stress. Choi and Ahn (2009) demonstrated that H_2O_2-induced osteoblast dysfunction was associated with oxidative damage to proteins and lipids in MC3T3-E1 cells and suggested the ability of dehydrocostus lactone to protect against oxidative stress induced inhibition of osteoblastic differentiation. A study revealed that costunolide-induced osteoblast differentiation was regulated by ATF4-dependent heme oxygenase-1 expression (Jeon, Kim, Kim, & Jang, 2017).

2.4. Antimicrobial activity

Costunolide showed significant antifungal activity. The MIC values of costunolide were as follows: 62.5 μg/mL against *Trichophyton mentagrophytes*, 62 μg/mL against *T. simii*, 31.25 μg/mL against *T. rubrum* 296, 62.5 μg/mL against *T. rubrum* 57, 125 μg/mL against *Epidermophyton floccosum*, 250 μg/mL against *Scopulariopsis* sp., 250 μg/mL against *Aspergillus niger*, 125 μg/mL against *Curvulari lunata*, 250 μg/mL against *Magnaporthe grisea*. Dehydrocostus lactone exhibited potent growth inhibitory and bactericidal activity toward five strains resistant to amoxicillin, clarithromycin, metronidazole, or tetracycline, indicating that dehydrocostus lactone and the antibiotics do not share a common mode of action (Chen, Zhang, Li, & Wang, 2018). Global efforts to reduce the level of antibiotics justify further studies on the extractive-derived materials containing dehydrocostus lactone as potential antibacterial products or a lead molecule for the prevention or eradication of drug-resistant *H. pylori*.

2.5. Others

Yang, Chang, Li, & Chen, 2019 found that *A. lappa* significantly decreased the mucosal damage index in SD rats induced by ethyl alcohol, and pyloric ligation. Costunolide and dehydrocostus lactone inhibit blood ethanol elevation and a-methyl or a-methylene group was essential to show inhibiting effect of ethanol absorption. Costunolide and dehydrocostus lactone decreased spontaneous motility induced by both methamphetamine and apomorphine. They can be considered as neuroleptics by resemblance of their pharmacological activities to chlorpromazine (Matsuda et al., 2003; Obaya & Sedivy, 2002; Shay & Wright, 1996). Guo et al. (2014) indicated that *A. lappa* extraction played a spasmolytic role in gastrointestinal motility, which was probably mediated through the inhibition of muscarinic receptors, serotonin receptors, and calcium influx. The presence of cholinergic and calcium antagonist constituents may be the compatibility of costunolide and dehydrocostus lactone (Pyun, Kang, Seo, & Lee, 2018). All of these results provide a pharmacological basis for its clinical involving in the gastrointestinal tract.

Costunolide promoted hair growth *in vitro* and *in vivo* by regulating the amount of growth factors and the activity of cellular responses through coordination of the WNT-β-catenin, hedgehog-Gli, and TGF-β1-Smad pathways (Lee et al., 2018). As has been discussed in earlier sections, pharmacological activities of costunolide and dehydrocostus lactone as well as the possible anti-cancer mechanisms of costunolide and dehydrocostus lactone have presented in Fig. 2 (Liu et al., 2011).

The traditional functions of *A. lappa* include promoting *Qi* circulation to relieve pain, invigorating spleen to relieve food stagnation, expelling dampness to remove fever and so on, which is also consistent with the pharmacological activities of the active ingredients of *A. lappa* mentioned above in the aspect of traditional Chinese medicine syndrome differentiation and treatment (Whipple et al., 2013). Under the theory of traditional Chinese medicine, for example, inflammation may be caused by deficiency of *Qi*, blood stasis or dampness and heat, while *A. lappa* itself can remove dampness and heat, reflecting the corresponding activities of effective components isolated from *A. lappa* (Noh, Kim, & Kim, 2014). In addition, studies on the activity of *A. lappa* against oxidative superoxide dismutase (SOD) showed that *A. lappa* can get rid of the damage caused by oxygen free radicals by increasing the endogenous SOD activity of the body, which may be one of the important anti-tumor mechanisms of *A. lappa*. Likewise, the anticancer effect of *A. lappa* is to improve the immunity of the organism (Zhuge et al., 2018).

3. Structure-activity relationship

Sesquiterpene lactones may exert their various biological activities by the interaction of their α-methylene-γ-butyrolactone with the thiol groups of biomacromolecules through Michael-addition. Sesquiterpene lactones [cynaropicrin, reynosin, and santamarine] inhibited TNF-α production in a dose-dependent manner. Chemical structures of cynaropicrin, reynosin, and santamarine were presented in Fig. 3 (Cho et al., 1998; Choi, Kim, & Lee, 2009). However, treatment with SH compounds such as L-cysteine, dithiothreitol and 2-mercaptoethanol abrogated the inhibitory effect of cynaropicrin on TNF-α production. Therefore, it was concluded that the principal inhibitory component of *A. lappa* was cynaropi-

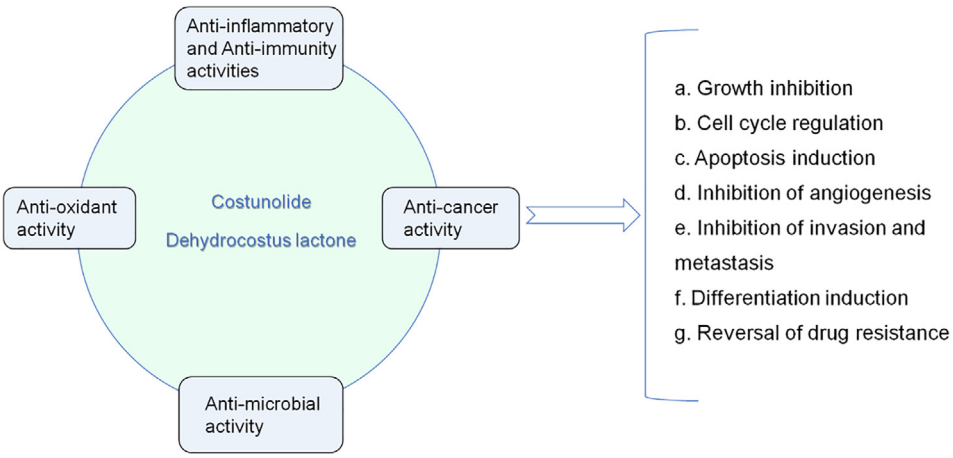

Fig. 2. Pharmacological activities and possible anti-cancer mechanisms of costunolide and dehydrocostus lactone.

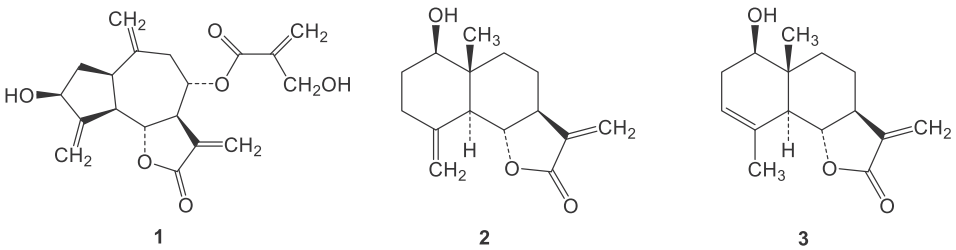

Fig. 3. Chemical structures of active monomers in the extractive and semi-synthetic analogues isolated from *A. lappa*. cynaropicrin (1), reynosin (2) and santamarine (3).

crin and its inhibitory effect was mediated through conjugation with SH-groups of target proteins (Yang, Kim, Lee, & Choi, 2011).

Choodej et al. (2018) isolated a number of sesquiterpenes, including costunolide and dehydrocostus lactone, which exhibited the highest efficiency in decreasing TNF-α levels, with IC_{50} values of 2.05 and 2.06 μmol/L, respectively. As the major component, costunolide, was used as the starting material for synthesis. The 10-membered ring of this type of compound has been shown to be highly prone to cyclization to a fused 6,6-bicyclic ring under acidic condition, providing a eudesmanolide-type sesquiterpene. A few of derivatives derived from costunolide as well as eudesmanolide-type sesquiterpene synthesizing from costunolide were exhibited in Fig. 4 (Choodej et al., 2018). Both compounds 1 and 2, which had no α-methylene-γ-butyrolactone moiety in their structure, did not show any detectable activity even at the highest concentration tested (50 μmol/L), whereas compound 3 showed active TNF-α inhibition. Based on the results above, the α-methy lene-γ-butyrolactone moiety was essential for the anti-inflammatory effect on TNF-α secretion in activated macrophages. Among the semi-synthetic analogues, compounds **4** and **5** showed the most potent activity with IC_{50} values of 1.84 and 1.97 μmol/L, respectively. Compounds **4** and **5** showed the most potent inhibitory activity among all compounds, comparable to the parent compound —costunolide. What's more, compound **4** did not show any significant cytotoxicity at the concentrations tested and was markedly less toxic than costunolide, whereas the cytotoxicity of compound **5** was comparable to that of costunolide, indicating that the epoxide derivative 4 might represent a lead compound for further anti-TNF-α therapies, owing to its potent activity and reduced toxicity. The results above indicated that α-methylene-γ-butyrolac tone is a crucial building block of many natural products exhibiting diverse biological activities including anti-inflammatory activity, hence it was considered important to retain this structural moiety (Tian et al., 2017). The summary of structure–activity relationship of the eudesmanolide skeleton for anti-TNF-α activity was listed in Fig. 5 (Wang et al., 2012).

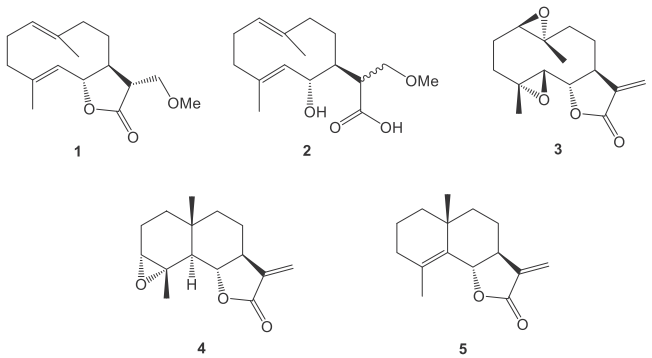

Fig. 4. Chemical structures of several sesquiterpene analogues derived from costunolide (compounds **1**–**3** were derivatives prepared by methoxylation, basic hydrolysis, and epoxidation of costunolide; compounds **4**–**5** were semi-synthetic analogues synthesized by costunolide).

The sesquiterpene lactones class of natural products displays a diverse array of biological activities due to the presence of the α-methylene-γ-butyrolactone (Parker, Kavallaris, & McCarroll, 2014). However, clinical translation of this class has been hampered by poor aqueous solubility and non-selective binding as a michael acceptor at undesired targets (Hasson, 2018). A prodrug approach has been developed to overcome these problems in which an amine is added into the α-methylene-γ-butyrolactone. Similar biological activities and mechanisms of action between the amino-adducts and the parent sesquiterpene lactones have been continuously demonstrated across helenalin, ambrosin, costunolide, saussureamines, alantolactone, and parthenolide, of which costunolide, saussureamines were isolated from the roots of *A. lappa* (Fang, Li, Wu, Gui, & Shen, 2019). The findings suggested that the amino-derivatives of sesquiterpene lactones can increase solubility and improve the selective binding as a Michael acceptor.

4. Pharmacokinetic and toxicological study

Pharmacokinetic and toxicological studies are very important to deeply elucidate the mechanism of pharmaceutical action. Studies demonstrated that costunolide and dehydrocostus lactone showed similar pharmacological activities (Eliza, Daisy, Ignacimuthu, & Duraipandiyan, 2009). Study from Hu et al. (2011) also revealed that pharmacokinetics parameters of costunolide and dehydrocostus lactone were similar too, which was showed in Table 2.

After oral administration of 0.125 g/kg mixed solution (containing 0.025 g costunolide and 0.1 g dehydrocostus lactone) to rats, peak concentrations of costunolide and dehydrocostus lactone were 24 and 63 μg/L (C_{max}) reached 9.0 and 6.0 h (T_{max}), respectively. The half-life and area under plasma concentration (area under the concentration–time curve 0–48) were found to be 4.97 and 5.44 h, and 330 and 1090 μg/L/h, respectively. Blood drug concentration vs time profiles were all fitted in a one compartment model. The corresponding coefficients were 0.927 and 0.954, respectively. And the pharmacokinetic results were reasonable and similar (Yang, Kim, Lee, & Choi, 2011).

Furthermore, in order to promote the development of the pharmacokinetic of traditional Chinese medicine, Zhang et al. (2015) administrated the dose of 2 g/kg herb extract which containing 0.015 g costunolide and 0.072 g dehydrocostus lactone to rats. The study found that the time to reach peak concentration (T_{max}) of costunolide and dehydrocostus lactone was the same at 12 h and peak concentration (C_{max}) attained to 0.02 and to 0.49 μg/L respectively. They also studied the pharmacokinetics on costunolide and dehydrocostus lactone after administration of traditional Chinese medicine Weichang'an Pills. In the blood samples, costunolide and dehydrocostus lactone showed a good linearity within concentration ranges 0.7–769.7, 2.51–956 μg/L, respectively. The results of precision, stability and recovery experiences proved the stability and reliability of the plasma concentration

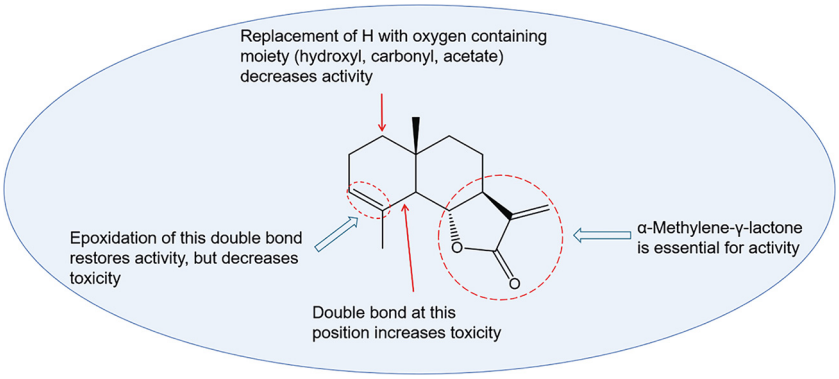

Fig. 5. Summary of structure–activity relationship of eudesmanolide skeleton for anti-TNF-α activity.

determination method. After the oral administration, the concentrations of costunolide and dehydrocostus lactone in plasma increased with the increase in dose, with maximum time between 10.65 and 12.98 h, maximum concentration of costunolide and dehydrocostus lactone between 3.750 and 5.450, 15.34–44.52 μg/L, respectively. The *in vivo* adsorption of costunolide and dehydrocostus lactone conformed to the one-compartment model, with a longer time to attain the peak plasma concentrations (Hao, Zhao, Gao, Xu, & Liu, 2010; Liu, Xiao, Peng, & Song, 2009; Qureshi & Enbergs, 2007).

The pharmacokinetic parameters of dehydrocostus lactone and costunolide displayed the significant changes with oral administration of Weichang'an Pills and extract of *A. lappa* compared with oral administration of the monomers (Table 2). The T_{max} was delayed from 9 h to 12 h for costunolide and 6 h to 12 h for dehydrocostus lactone, which could be influenced by other constituents in the herb extract of *A. lappa* and Weichang'an Pills, including several other sesquiterpene lactones, monoterpene, triterpene, et al, which were most important to provide the data for the reaches of the pharmacokinetic of traditional Chinese medicine (Dae & Bu, 2019).

Besides, Gao, Li, Chen, Xu, Chen, and Hu (2011) showed the study that costunolide and dehydrocostus lactone could bind to human serum albumin through the Van der Waals' force and hydrogen bond formation by molecule modeling, atomic force microscope, and different optical techniques including Fluorescence, Circular dichroism, Fourier transform infrared spectroscopy, and Raman spectra (Gismondi et al., 2013). Moreover, the study has found that the binding constant of human serum albumin with costunolide is stronger than that of human serum albumin with dehydrocostus lactone, which may be useful to study the pharmacological differences between costunolide and dehydrocostus lactone (Choi et al., 2013).

The extractive showed anticancer activity due to the presence of the α-methylene-γ-butyrolactone. However, the non-selective binding as a Michael acceptor at undesired targets would also lead to potential hepatotoxic activity. Zhao, Lu, Hu, and Whang (2012) screened the potential hepatotoxic components in *A. lappa*. The potential hepatotoxic components were screened using HepG2 cells labeled with fluorescein diacetate from 25 fractions of *A. lappa*, in which the hepatotoxic compounds were further identified with GC–MS. The result suggested that dehydrocostus lactone, santamarine (or magnolialide), reynosin, α-costol and elemol were potential hepatotoxic compounds in *A. lappa*. Obscurely, α-costol and elemol, two compounds of the extractive with no α-methy lene-γ-butyrolactone moiety, showed hepatotoxic activity. Hence, the mechanism of hepatotoxic activity of α-costol and elemol is needed in the future studies (Lin, Peng, & Su, 2015).

5. Discussion

Substantial studies showed that sesquiterpene lactones were the effective components in *A. lappa*. And the extractive have been reported have anti-inflammatory, antiviral, and anti-tumor properties, anti-ulcer and other effects (Chen, Chou, Lee, Wang, & Yeh, 1995). The main pharmacological effects and corresponding active components isolated from *A. lappa* are shown in Table 3. Costunolide and dehydrocostus lactone, two main bioactive compounds of *A. lappa*, have been studied most, including pharmacological activities, structure–activity relationship, pharmacokinetic as well as toxicology. The extractive may exert their various biological activities, especially for its anti-cancer activity, by the interaction of their α-methylene-γ-butyrolactone with the thiol groups of biomacro molecules through Michael-addition (Hua, Zhang, Zhang, Sun, Cui, & Li, 2016; Kim et al., 1994; Matsuda et al., 2000; Tabata et al., 2015; Zimmermann et al., 2014). Besides, studies demonstrated that the extractive, owing to α-methylene-γ-butyro lactone, showed similar pharmacological activities and pharmacokinetics parameters. The pharmacokinetic parameters of dehydrocostus lactone and costunolide may be influenced by other constituents, including several other sesquiterpene lactones, monoterpene, triterpene, et al. Generally speaking, the extractive uphold prospective candidates for modern drugs development

Table 2
Pharmacokinetics study on costunolide and dehydrocostus lactone.

Drugs	Species	Route	C_{max}/ (μg·L^{-1})		T_{max} /h	
			costunolide	Dehydrocostus lactone	costunolide	Dehydrocostus lactone
0.125 g/kg mixed solution (containing 0.025 g costunolide and 0.1 g dehydrocostus lactone)	rat	oral	24	63	9	6
2 g/kg herb extract (containing 0.015 g costunolide and 0.072 g dehydrocostus lactone)	rat	oral	0.02	0.49	12	12
0. 8 g/kg extract of Weichang'an pills	rat	oral	5.45	44.52	11.52	12.39

Table 3
Main pharmacological effects and corresponding active components.

Pharmacological effects	Corresponding active components
Anti-inflammatory effect	cynaropicrin, reynosin, santamarine, dehydrocostus lactone, costunolide, saussureamine A, saussureamine B
Anti-tumor effect	β-peltatin, lignans ramification, costunolide, dehydrocostus lactone, 1β-hydroxy arbusculin A, reynosin, isodihydroxylan lactone
Cholagogic effect	acetone extracts, costunolide, ethanol extracts
Anti-gastric ulcer effect	saussureamines A, saussureamines B, saussureamines C, dehydrocostus lactone, costunolide
Spasmolysis and analgesia	total lactones, dihydrocostunolide, dihydrocostuslactone, dehydrocostus lactone, costunolide, methanol extracts
Dilating blood vessels and reducing blood pressure	total lactones, alkaloid, costuslactone, dihydrocostuslactone, 12-methoxy dihydrocostus lactone, volatile oil, dehydrocostus lactone, costunolide

(Duraipandiyan, Al-Harbi, Ignacimuthu, & Muthukumar, 2012; Hsu et al., 2011; Yang, Zhang, Yang, Le, & Chen, 2016).

However, clinical translation of this class has been hampered by poor aqueous solubility, non-selective binding as a Michael acceptor at undesired targets (Yoshikawa et al., 2000). A prodrug approach has been developed to increase aqueous solubility of sesquiterpene lactones, in which an amine is added into the α-m ethylene-γ-butyrolactone. Besides, the pharmacokinetic and toxicological studies of the extractive are limited, which cannot provide effective data support for its safety of being new clinical drug (Eliza, Daisy, & Ignacimuthu, 2010).

In order to promote new drug development from the extractive for treating various diseases, further research is needed to develop the pharmacokinetic and toxicological studies of the extractive. Meanwhile, future research can also be concentrated on the structure transformation, dosage form, route of administration and even the target proteins of the extractive. Considering the therapeutic value of sesquiterpene lactones isolated from *A. lappa*, it would be interesting to further examine the effects of costunolide and dehydrocostus lactone in various other animal models to reveal the subacute and chronic toxicities, detailed elucidation of molecular mechanisms of action, and structural modifications to develop new therapeutics based on sesquiterpene lactones or their derivatives. In a word, taking these above concerns into account, we can explore and utilize the extractive well in the form of the clinical drug.

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References

Abd Eldaim, M. A., Tousson, E., El Sayed, I. E. T., & Awd, W. M. (2019). Ameliorative effects of *Saussurea lappa* root aqueous extract against Ethephon-induced reproductive toxicity in male rats. *Environmental Toxicology, 34*(2), 150–159.

Bocca, C., Gabriel, L., Bozzo, F., & Miglietta, A. (2004). A sesquiterpene lactone, costunolide, interacts with microtubule protein and inhibits the growth of MCF-7 cells. *Chemico-biological Interactions, 147*(1), 79–86.

Cai, H., He, X., & Yang, C. (2018). Costunolide promotes imatinib-induced apoptosis in chronic myeloid leukemia cells via the Bcr/Abl-Stat5 pathway. *Phytotherapy Research, 32*(9), 1764–1769.

Chen, H. C., Chou, C. K., Lee, S. D., Wang, J. C., & Yeh, S. F. (1995). Active compounds formsaussurea lappa clarks that suppress hepatitis B virus surface angtigengene expression in human hepatoma cells. *Antiviral Research, 27*, 99–109.

Chen, J., Chen, B., Zou, Z., Li, W., Zhang, Y., Xie, J., et al. (2017). Costunolid enhances doxorubicin-induced apoptosis in prostate cancer cells via activated mitogen-activated protein kinases and generation of reactive oxygen species. *Oncotarget, 8*(64), 107701–107715.

Chen, Z., Zhang, D., Li, M., & Wang, B. (2018). Costunolide ameliorates lipoteichoic acid-induced acute lung injury via attenuating MAPK signaling pathway. *International Immunopharmacology, 61*, 283–289.

Cheong, C. U., Yeh, C. S., Hsieh, Y. W., Lee, Y. R., Lin, M. Y., Chen, C. Y., et al. (2016). Protective effects of costunolide against hydrogen peroxide-induced injury in PC12 cells. *Molecules, 21*(7), 898.

Choi, E. J., & Ahn, W. S. (2009). Antiproliferative effects of dehydrocostus lactone through cell cycle arrest and apoptosis in human ovarian cancer SK-OV-3 cells. *International Journal of Molecular Medicine, 23*, 211–216.

Choi, E. M., Kim, G. H., & Lee, Y. S. (2009). Protective effects of dehydrocostus lactone against hydrogen peroxide-induced dysfunction and oxidative stress in osteoblastic MC3T3-E1 cells. *Toxicology In Vitro, 23*(5), 862–867.

Choi, H. G., Lee, D. S., Li, B., Choi, Y. H., Lee, S. H., & Kim, Y. C. (2012a). Santamarin, a sesquiterpene lactone isolated from *Saussurea lappa*, represses LPS-induced inflammatory responses via expression of heme oxygenase-1 in murine macrophage cells. *International Immunopharmacology, 13*(3), 271–279.

Choi, S. H., Im, E., Kang, H. K., Lee, J. H., Kwak, H. S., Bae, Y. T., et al. (2005). Inhibitory effects of costunolide on the telomerase activity in human breast carcinoma cells. *Cancer Letters, 227*(2), 153–162.

Choi, Y. K., Cho, S. G., Woo, S. M., Yun, Y. J., Jo, J., Kim, W., et al. (2013). *Saussurea lappa* clarke-derived costunolide prevents TNFα-induced breast cancer cell migration and invasion by inhibiting NF-κB activity. *Evidence-based Complementary and Alternative Medicine, 936257*.

Choi, Y. K., Seo, H. S., Choi, H. S., Choi, H. S., Kim, S. R., Shin, Y. C., et al. (2012b). Induction of Fas-mediated extrinsic apoptosis, p21WAF1-related G2/M cell cycle arrest and ROS generation by costunolide in estrogen receptor-negative breast cancer cells, MDA-MB-231. *Molecular and Cellular Biochemistry, 363*(1-2), 119–128.

Cho, J. Y., Park, J., Yoo, E. S., Baik, K. U., Jung, J. H., Lee, J., et al. (1998). Inhibitory effect of sesquiterpene lactones from *Saussurea lappa* on tumor necrosis factor-alpha production in murine macrophage-like cells. *Planta Medica, 64*(7), 594–597.

Choodej, S., Pudhom, K., & Mitsunaga, T. (2018). Inhibition of TNF-α-induced inflammation by sesquiterpene lactones from *Saussurea lappa* and semi-synthetic analogues. *Planta Medica, 84*(05), 329–335.

Chun, J., Choi, R. J., Khan, S., Lee, D. S., Kim, Y. C., Nam, Y. J., et al. (2012). Alantolactone suppresses inducible nitric oxide synthase and cyclooxygenase-2 expression by down-regulating NF-κB, MAPK and AP-1 via the MyD88 signaling pathway in LPS-activated RAW 264.7 cells. *International Immunopharmacology, 14*(4), 375–383.

Committee of National Pharmacopoeia. (2015). Pharmacopoeia of People's Republic of China. Chemical Industry Press, p. 185.

Dae, Y. K., & Bu, Y. C. (2019). Costunolide—a bioactive sesquiterpene lactone with diverse therapeutic potential. *International Journal of Molecular Sciences, 20*, 2926.

Duraipandiyan, V., Al-Harbi, N. A., Ignacimuthu, S., & Muthukumar, C. (2012). Antimicrobial activity of sesquiterpene lactones isolated from traditional medicinal plant, *Costus speciosus* (Koen ex. Retz.) Sm. BMC. *Journal of Alternative and Complementary Medicine, 12*, 13.

Eliza, J., Daisy, P., Ignacimuthu, S., & Duraipandiyan, V. (2009). Normo-glycemic and hypolipidemic effect of costunolide isolated from *Costus speciosus* (Koen ex. Retz.) Sm. in streptozotocin-induced diabetic rats. *Chemico-Biological Interactions, 179*(2-3), 329–334.

Eliza, J., Daisy, P., & Ignacimuthu, S. (2010). Antioxidant activity of costunolide and eremanthin isolated from *Costus speciosus* (Koen ex. Retz) Sm. *Chemico-Biological Interactions, 188*(3), 467–472.

Fang, Y., Li, J., Wu, Y., Gui, J., & Shen, Y. (2019). Costunolide inhibits the growth of OAW42-A multidrug-resistant human ovarian cancer cells by activating apoptotic and autophagic pathways, production of reactive oxygen species (ROS), Cleaved Caspase-3 and cleaved Caspase-9. *Medical Science Monitor, 25*, 3231–3237.

Gao, W. H., Li, N. N., Chen, G. P., Xu, Y. P., Chen, Y. W., Hu, S. L., et al. (2011). Binding studies of costunolide and dehydrocostus lactone with HAS by spectroscopy and atomic force microscopy. *Journal of Luminescence*, 131, 2063–2071.

Gismondi, A., Canuti, L., Impei, S., Marco, G., Kenzo, M., Colizzi, V., et al. (2013). Antioxidant extracts of African medicinal plants induce cell cycle arrest and differentiation in B16F10 melanoma cells. *International Journal of Oncology*, 43, 956–964.

Guo, H., Zhang, J., Gao, W., Qu, Z., & Liu, C. (2014). Gastrointestinal effect of methanol extract of *Radix Aucklandiae* and selected active substances on the transit activity of rat isolated intestinal strips. *Pharmaceutical Biology, 52*(9), 1141–1149.

Hao, L. J., Zhao, F., Gao, Z. T., Xu, H., & Liu, K. (2010). Inhibitory effects of sesquiterpenes from *Saussurea lappa* on the vascular endothelial growth factor. *Natural Product Research, 22*, 687–691.

Hasson, S. S., HAl-Shubi, A. S., Al-Busaidi, J. Z., Al-Balushi, M. S., Hakkim, F. L., Rashan, L., et al. (2018). Potential of *Aucklandia lappa* Decne ethanolic extract to trigger apoptosis of human T47D and Hela cells. *Asian Pacific Journal of Cancer Prevention, 19*(7), 1917–1925.

Hsu, J. L., Pan, S. L., Ho, Y. F., Hwang, T. L., Kung, F. L., & Guh, J. H. (2011). Costunolide induces apoptosis through nuclear calcium^{2+} overload and DNA damage response in human prostate cancer. *World Journal of Urology, 185*(5), 1967–1974.

Hua, P., Zhang, G., Zhang, Y., Sun, M., Cui, R., Li, X., et al. (2016). Costunolide induces G1/S phase arrest and activates mitochondrial-mediated apoptotic pathways in SK-MES 1 human lung squamous carcinoma cells. *Oncology Letters*, 11(4), 2780-2786.

Hu, F. D., Feng, S. L., Wu, Y. Q., Bi, Y. Y., Wang, C. M., & Li, W. (2011). Quantitative analysis of costunolide and dehydrocostus lactone in rat plasma by ultraperformance liquid chromatography-electrospray ionization-mass spectrometry. *Biomedical Chromatography, 25*(5), 547–554.

Hung, J. Y., Hsu, Y. L., Ni, W. C., Tsai, Y. M., Yang, C. J., Kuo, P. L., et al. (2010). Oxidative and endoplasmic reticulum stress signaling are involved in dehydrocostus lactone-mediated apoptosis in human non-small cell lung cancer cells. *Lung Cancer, 68*(3), 355–365.

Jeong, G. S., Pae, H. O., Jeong, S. O., Kim, Y. C., Kwon, T. O., Lee, H. S., et al. (2007). The alpha-methylene-gamma-butyrolactone moiety in dehydrocostus lactone is responsible for cytoprotective heme oxygenase-1 expression through activation of the nuclear factor E2-related factor 2 in HepG2 cells. *European Journal of Pharmacology, 565*, 37–44.

Jeon, W. J., Kim, K. M., Kim, E. J., & Jang, W. G. (2017). Costunolide increases osteoblast differentiation via ATF4-dependent HO-1 expression in C3H10T1/2 cells. *Life Sciences, 178*, 94–99.

Kanno, S., Kitajima, Y., Kakuta, M., Osanai, Y., Kurauchi, K., Ujibe, M., et al. (2008). Costunolide-induced apoptosis is caused by receptor-mediated pathway and inhibition of telomerase activity in NALM-6 cells. *Biological and Pharmaceutical Bulletin, 31*(5), 1024–1028.

Kassuya, C. A., Cremoneze, A., Barros, L. F., Simas, A. S., Lapa, F. R., Mello-Silva, R., et al. (2009). Antipyretic and anti-inflammatory properties of the ethanolic extract, dichloromethane fraction and costunolide from *Magnolia ovate* (Magnoliaceae). *Journal of Ethnopharmacology, 124*(3), 369–376.

Khan, Q. A., Pediaditakis, P., Malakhau, Y., Esmaeilniakooshkghazi, A., Ashkavand, Z., Sereda, V., et al. (2018). CHIP E3 ligase mediates proteasomal degradation of the proliferation regulatory protein ALDH1L1 during the transition of NIH3T3 fibroblasts from G0/G1 to S-phase. *PLoS ONE, 13*(7), e0199699.

Kim, E. J., Lim, S. S., Park, S. Y., Shin, H. K., Kim, J. S., & Park, J. H. Y. (2008). Apoptosis of DU145 human prostate cancer cells induced by dehydrocostus lactone isolated from the root of *Saussurea lappa*. *Food and Chemical Toxicology, 46*(12), 3651–3658.

Kim, N., Piatyszek, M., Prowse, K., Harley, C., West, M., Ho, P., et al. (1994). Specific association of human telomerase activity with immortal cells and cancer. *Science, 266*(5193), 2011–2015.

Kim, Y. E., Choi, H. C., Nam, G., & Choi, B. Y. (2018). Costunolide promotes the proliferation of human hair follicle dermal papilla cells and induces hair growth in C57BL/6 mice. *Journal of Cosmetic Dermatology, 18*(1), 414–421.

Ko, S. G., Kim, H. P., Jin, D. H., Bae, H. S., Kim, S. H., Park, C. H., et al. (2005). *Saussurea lappa* induces G2-growth arrest and apoptosis in AGS gastric cancer cells. *Cancer Letters, 220*(1), 11–19.

Lee, B. K., Park, S. J., Nam, S. Y., Kang, S., Hwang, J., Lee, S. J., et al. (2018). Anti-allergic effects of sesquiterpene lactones from *Saussurea costus* (Falc.) Lipsch. determined using *in vivo* and *in vitro* experiments. *Journal of Ethnopharmacology, 213*, 256–261.

Lee, H. K., Song, H. E., Lee, H. B., Kim, C. S., Koketsu, M., Thi My Ngan, L., et al. (2014). Growth inhibitory, bactericidal, and morphostructural effects of dehydrocostus lactone from *Magnolia sieboldii* leaves on antibiotic-susceptible and -resistant strains of Helicobacter pylori. *PLoS One, 9*(4), e95530.

Lim, H. S., Ha, H., Lee, M. Y., Jin, S. E., Jeong, S. J., Jeon, W. Y., et al. (2014). *Saussurea lappa* alleviates inflammatory chemokine production in HaCaT cells and house dust mite-induced atopic-like dermatitis in Nc/Nga mice. *Food and Chemical Toxicology, 63*, 212–220.

Lin, X. J., Peng, Z. X., Fu, X. H., Liu, C. Y., Xu, Y., Ji, W. D., et al. (2016). Volatile oil from *Saussurea lappa* exerts antitumor efficacy by inhibiting epithelial growth factor receptor tyrosine kinase-mediated signaling pathway in hepatocellular carcinoma. *Oncotarget, 7*(48), 79761–79773.

Lin, X. J., Peng, Z. X., & Su, C. Q. (2015). Potential anti-cancer activities and mechanisms of costunolide and dehydrocostus lactone. *International Journal of Molecular Sciences, 16*(5), 10888–10906.

Liu, C. X., Xiao, P. G., Peng, Y., & Song, N. N. (2009). Challenges in research and development of traditional Chinese medicines. *Chinese Herbal Medicines, 1*(1), 1–28.

Liu, C. Y., Chang, H. S., Chen, I. S., Chen, C. J., Hsu, M. L., Fu, S. L., et al. (2011). Costunolide causes mitotic arrest and enhances radiosensitivity in human hepatocellular carcinoma cells. *Radiation Oncology Investigations, 6*(1), 56. https://doi.org/10.1186/1748-717X-6-56.

Lohberger, B., Rinner, B., Stuendl, N., Kaltenegger, H., Steinecker-Frohnwieser, B., Bernhart, E., et al. (2013). Sesquiterpene lactones downregulate G2/M cell cycle regulator proteins and affect the invasive potential of human soft tissue sarcoma cells. *PLoS ONE, 8*(6), e66300.

Matsuda, H., Kagerura, T., Toguchida, I., Ueda, H., Morikawa, T., & Yoshikawa, M. (2000). Inhibitory effects of sesquiterpenes from bay leaf on nitric oxide production in lipopolysaccharide-activated macrophages: Structure requirement and role of heat shock protein induction. *Life Sciences, 66*(22), 2151–2157.

Matsuda, H., Toguchida, I., Ninomiya, K., Kageura, T., Morikawa, T., & Yoshikawa, M. (2003). Effects of sesquiterpenes and amino acid-sesquiterpene conjugates from the roots of *Saussurea lappa* on inducible nitric oxide synthase and heat shock protein in lipopolysaccharide-activated macrophages. *Bioorganic and Medicinal Chemistry Letters, 11*(5), 709–715.

Noh, H. J., Kim, K. A., & Kim, K. C. (2014). p53 down-regulates SETDB1 gene expression during paclitaxel induced-cell death. *Biochemical and Biophysical Research Communications, 446*(1), 43–48.

Obaya, A. J., & Sedivy, J. M. (2002). Regulation of cyclin-Cdk activity in mammalian cells. *Cellular and Molecular Life Sciences, 59*(1), 126–142.

Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2007). *Saussurea costus*: Botanical, chemicaland pharmacological review of an Ayurvedic medicinal plant. *Journal of Ethnopharmacology, 110*(3), 379–390.

Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelvespecies of Indian medicinal plants. *African Journal of Microbiology Research, 10*, 175–181.

Parker, A. L., Kavallaris, M., & McCarroll, J. A. (2014). Microtubules and their role in cellular stress in cancer. *Frontiers in Oncology, 4*, 153.

Park, E., Song, J. H., Kim, M. S., Park, S. H., & Kim, T. S. (2016). Costunolide, a sesquiterpene lactone, inhibits the differentiation of pro-inflammatory CD4(+) T cells through the modulation of mitogen-activated protein kinases. *International Immunopharmacology, 40*, 508–516.

Pavan Kumar, C., Devi, A., Ashok Yadav, P., Rao Vadaparthi, R., Shankaraiah, G., Sowjanya, P., et al. (2016). "Click" reaction mediated synthesis of costunolide and dehydrocostus lactone derivatives and evaluation of their cytotoxic activity. *Journal of Asian Natural Products Research, 18*(11), 1063–1078.

Peng, Z. X., Wang, Y., Fan, J. H., Lin, X. J., Liu, C. Y., Xu, Y., et al. (2017). Costunolide and dehydrocostus lactone combination treatment inhibit breast cancer by inducing cell cycle arrest and apoptosis through c-Myc/p53 and AKT/14-3-3 pathway. *Scientific Reports, 7*, 41254.

Pitchai, D., Roy, A., & Banu, S. (2014). *In vitro* and *in silico* evaluation of NF-κB targeted costunolide action on estrogen receptor-negative breast cancer cells-acomparison with normal breast cells. *Phytotherapy Research, 28*(10), 1499–1505.

Pyun, H., Kang, U., Seo, E. K., & Lee, K. (2018). Dehydrocostus lactone, a sesquiterpene from *Saussurea lappa* Clarke, suppresses allergic airway inflammation by binding to dimerized translationally controlled tumor protein. *Phytomedicine, 43*, 46–54.

Qureshi, A. S., & Enbergs, H. (2007). Effects of *Saussurea lappa* roots extract in ethanol on leukocyte phagocytic activity, lymphocyte proliferation and interferon-gamma (IFN-γ). *Pakistan Journal of Pharmaceutical Sciences, 20*(3), 175.

Rasul, A., Bao, R., Malhi, M., Zhao, B., Tsuji, I., Li, J., et al. (2013). Induction of apoptosis by costunolide in bladder cancer cells is mediated through ROS generation and mitochondrial dysfunction. *Molecules, 18*(2), 1418–1433.

Saraswati, S., Alhaider, A. A., & Abdelgadir, A. M. (2018). Costunolide suppresses an inflammatory angiogenic response in a subcutaneous murine sponge model. *Journal of Pathology, Microbiology and Immunology, 126*(3), 257–266.

Shay, Jerry W., & Wright, Woodring E. (1996). Telomerase activity in human cancer. *Current Opinion in Oncology, 8*(1), 66–71.

Spencer, N. Y., Yang, Z. H., Sullivan, J. C., Klein, T., Stanton, R. C., & Jandeleit-Dahm, K. (2018). Linagliptin unmasks specific antioxidant pathways protective against albuminuria and kidney hypertrophy in a mouse model of diabetes. *PLoS One, 13*(7), e0200249.

Seo, C. S., Lim, H. S., Jeong, S. J., & Shin, H. K. (2015). Anti-allergic effects of sesquiterpene lactones from the root of Aucklandia lappa Decne. *Molecular Medicine Reports*, 12, 7789–7795.

Tabata, K., Nishimura, Y., Takeda, T., Kurita, M., Uchiyama, T., & Suzuki, T. (2015). Sesquiterpene lactones derived from *Saussurea lappa* induce apoptosis and inhibit invasion and migration in neuroblastoma cells. *Journal of Pharmacological Sciences, 127*(4), 397–403.

Tian, X., Song, H. S., Cho, Y. M., Park, B., Song, Y. J., Jang, S., et al. (2017). Anticancer effect of *Saussurea lappa* extract via dual control of apoptosis and autophagy in prostate cancer cells. *Medicine (Baltimore), 96*(30), e7606.

Wang, C. Y., Tsai, A. C., Peng, C. Y., Chang, Y. L., Lee, K. H., Teng, C. M., et al. (2012). Dehydrocostuslactone suppresses angiogenesis *in vitro* and *in vivo* through inhibition of Akt/GSK-3β and mTOR signaling pathways. *PLoS ONE, 7*(2), e31195.

Wang, Y. N., Zhang, X., Zhao, L. L., Shi, M. Y., Wei, Z. K., Yang, Z. T., et al. (2017). Costunolide protects lipopolysaccharide/d-galactosamine–induced acute liver injury in mice by inhibiting NF-κB signaling pathway. *Journal of Surgical Research, 220*, 40–45.

Wei, H., Peng, Y., Ma, G. X., Xu, L. J., & Xiao, P. G. (2012). Research progress on active components and pharmacological effects of *Aucklandia lappa*. *Chinese Traditional and Herbal Drugs, 43*(3), 613–620.

Whipple, R. A., Vitolo, M. I., Boggs, A. E., Charpentier, M. S., Thompson, K., & Martin, S. S. (2013). Parthenolide and costunolide reduce microtentacles and tumor cell attachment by selectively targeting detyrosinated tubulin independent from NF-κB inhibition. *Breast Cancer Research, 15*, R83.

Xue, J. L., Zhang, X. P., & Chang, Q. S. (2015). Potential anti-cancer activities and mechanisms of costunolide and dehydrocostuslactone. *International Journal of Molecular Sciences, 16*, 10888–10906.

Yang, M. H., Chang, K. J., Li, B., & Chen, W. S. (2019). Arsenic trioxide suppresses tumor growth through antiangiogenesis via Notch signaling blockade in small-cell lung cancer. *Biomed Research International, 2019*, 1–9.

Yang, X., Zhang, X., Yang, S. P., Le, T., & Chen, B. (2016). Evaluation of *Aucklandia lappa* Decne extracts as antiulcer activity in animals. *Pakistan Journal of Pharmaceutical Sciences, 29*(5), 1695–1701.

Yang, Y. I., Kim, J. H., Lee, K. T., & Choi, J. H. (2011). Costunolide induces apoptosis in platinum-resistant human ovarian cancer cells by generating reactive oxygen species. *Gynecologic Oncology, 123*(3), 588–596.

Yin, H. Q., Qi, X. L., Hua, H. M., & Pei, Y. H. (2005). The chemical constituents from *Saussurea lappa* C.B Clarke. *Journal of Medicinal Chemistry, 15*(4), 217–220.

Yoshikawa, M., Shimoda, H., Uemura, T., Morikawa, T., Kawahara, Y., & Matsuda, H. (2000). Alcohol absorption inhibitors from bay leaf (*Laurus nobilis*): Structure-requirements of sesquiterpenes for the activity. *Bioorganic and Medicinal Chemistry, 8*(8), 2071–2077.

Zhang, J. Z., Hu, X., Gao, W. Y., Qu, Z., Guo, H. M., Liu, Z., et al. (2014). Pharmacokinetic study on costunolide and dehydrocostus lactone after oral administration of traditional medicine *Aucklandia lappa* Decne by LC/MS/MS. *Journal of Ethnopharmacology, 151*(1), 191–197.

Zhang, J. Z., Wang, L., Jin, Z. X., Qu, Z., Chen, Y. L., & Gao, W. Y. (2015). Pharmacokinetics study on costunolde and dehydrocostus lactone after administration of traditional Chinese medicine Weichang'an pills. *China Journal of Chinese Materia Medica, 40*(6), 1173–1178.

Zhao, X. P., Lu, L., Hu, B., & Whang, S. F. (2012). Screening and identifying hepatotoxic components in *Aucklandiae Radix* with GC-MS. *Journal of Zhejiang University Medical Sciences, 41*(01), 43–46.

Zhuge, W., Chen, R., Vladimir, K., Dong, X., Zia, K., Sun, X., et al. (2018). Costunolide specifically binds and inhibits thioredoxin reductase 1 to induce apoptosis in colon cancer. *Cancer Letters, 412*, 46–58.

Zimmermann, S., Fouché, G., De Mieri, M., Yoshimoto, Y., Usuki, T., Nthambeleni, R., et al. (2014). Structure-activity relationship study of sesquiterpene lactones and their semi-synthetic amino derivatives as potential antitrypanosomal products. *Molecules, 19*(3), 3523–3538.