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REVIEW

Arnicolide D: a multi-targeted anticancer sesquiterpene lactone—preclinical efficacy and mechanistic insights

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Abstract

Arnicolide D, a potent sesquiterpene lactone from *Centipeda minima*, has emerged as a promising anticancer candidate, demonstrating significant efficacy in inhibiting cancer cell proliferation, inducing apoptosis, and suppressing metastasis across various cancer models. This comprehensive study delves into the molecular underpinnings of Arnicolide D's anticancer actions, emphasizing its impact on key signaling pathways such as PI3K/AKT/mTOR and STAT3, and its role in modulating cell cycle and survival mechanisms. Quantitative data from preclinical studies reveal Arnicolide D's dose-dependent cytotoxicity against cancer cell lines, including nasopharyngeal carcinoma, triple-negative breast cancer, and human colon carcinoma, showcasing its broad-spectrum anticancer potential. Given its multifaceted mechanisms and preclinical efficacy, Arnicolide D warrants further investigation in clinical settings to validate its therapeutic utility against cancer. The evidence presented underscores the need for rigorous pharmacokinetic and toxicological studies to establish safe dosing parameters for future clinical trials.

Keywords *Centipeda minima* · Arnicolide D · Anti-cancer mechanisms · Apoptosis · PI3K/AKT/mTOR pathway · Sesquiterpene lactone, Src degradation

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Introduction

Cancer remains a leading cause of morbidity and mortality worldwide, with an urgent need for more effective and less toxic therapies. The exploration of natural compounds, particularly from medicinal herbs like *Centipeda minima*, offers a promising avenue for novel anticancer drug discovery (Sharma et al. 2022) (Cunha et al. 2023) (Housman et al. 2014). An amalgamation of potent traditional herbal medicines with conventional anticancer therapies can exhibit maximum clinical benefit and be the most suitable route for cancer treatment (Okem et al. 2023). Herbal medicines are considered natural therapies for the treatment of diseases with the advantages of minimal side effects, cost-effectiveness, and potent efficacy (Singh and Gohil 2024). There are a few patents filed on *C. minima* Arnicolide D for neuroprotection and anti-viral action against different influenza infections, including anti-H1N1, H5N1, and H9N2 subtypes, to activate the bombesin receptor subtype 3 (BRS3) which has different functions including controlling of energy homeostasis, feeding pattern in small animals, etc. In different parts of Asia, *C. minima* are used to treat headaches, piles, malaria, cold, conjunctivitis, rickets, sores, digestive disorders, clear the nostrils, treat cough, sinus infections, cold, allergies, asthma, etc., in folk-lore systems. In Ayurveda, various ancient texts mention different uses for this herb in different formulations, which include oil formulations, snuff powder, ghee-based, etc., to treat a range of ailments, including fever, skin diseases, cough, deworming, nasal catarrh, etc. Some studies report the anti-cancer activity of Arnicolide D extracted from *C. minima*, indicating its potential utility in treating breast, colon, and nasopharyngeal cancer. In nasopharyngeal carcinoma cell lines, the effect of Arnicolide D was extensively studied. Cell cycle arrest was induced by Arnicolide D, which modulates different signaling pathways leading to cell cycle arrest. The utility of Arnicolide D was also studied on prostate cancer, colon cancer, melanoma, etc., and the results were reported to be promising**.** Plant sources like *C. minima*, *Arnica montana*, and *Arnica acaulis* are reported to contain Arnicolide D. *C. minima* is a well-recognized herbal medicine with multiple chemically active components, which has been utilized to treat various diseases. Reported bioactive properties of *C. minima* include anti-cancer, antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective activities. It has reported therapeutic effects for many diseases, such as rhinitis and sinusitis, gynecological infections, colds, coughs, and asthma (Tang and Eisenbrand 1992; Guo et al. 2015; Yuan et al. 2016; Wang et al. 2017; Li et al. 2020b). From various confirmations, it is reported that the bioactive constituents of *C. minima* are sesquiterpenoids, flavonoids, sterols, and glycosides. Among them, sesquiterpene lactones dominate in *C. minima* species and are the main bioactive component reported to have potent anticancer effectiveness (Jia et al. 2021). Among the various lactones, Brevilin A and Arnicolide D are reported as the most pharmacologically active components identified (Wu et al. 2012; Huang et al. 2016; Liu et al. 2019a, c; Zhu et al. 2019a; Su et al. 2020), with potential anti-cancer effects against breast cancer (Qu et al. 2020), prostate cancer (Yao et al. 2022a), and nasopharyngeal carcinoma (Liu et al. 2019a) with different rates associated with drug concentration-dependent manner. Also, Arnicolide D has been reported to inhibit the NF-κB pathway in colon cancer cells (Huang et al. 2014). As mentioned, Arnicolide D (Yao et al. 2022a), the most active sesquiterpenoid of *C. minima* (Yao et al. 2022a), can modify the cell cycle, inhibit the PI3K/ AKT/TOR and STAT3 signaling, and effectively stimulates the caspase signaling. Arnicolide D inhibition against the cancerous cell feasibility depends on time and concentration. However, studies exploring the anti-cancer activities of Arnicolide D are very much confined to only limited reports in the literature. In addition, among the various sesquiterpene lactones present in *C. minima* – Arnicolide D has reportedly exhibited superior efficacy than Brevillin A – reported anticancer drug (Wu et al. 2012), concerning the anti-nasopharyngeal carcinoma effect (Lee et al. 2020b). Furthermore, the findings on the various anticancer effects of Arnicolide D provide a robust platform to prove its emergence as an efficient compound for developing novel anticancer drugs. However, the structure–activity relationships and molecular mechanisms related to the antitumor effect of Arnicolide D have not been reported. This review aims to synthesize current knowledge on herbal anticancer drugs, focusing on Arnicolide D from *C. minima*, to understand its mechanisms and potential in cancer therapy development.

Methodology

The primary objective of this study is to investigate the mechanisms of action of Arnicolide D in the context of cancer therapy; this entails a detailed exploration of the molecular pathways influenced by Arnicolide D and their subsequent impact on cancer cell proliferation, apoptosis, and metastasis. A literature search was conducted across various databases, including PubMed/MedLine, Scopus, TRIP Database, Web of Science, and Google Scholar, to identify relevant preclinical studies. The search strategy incorporated a combination of MeSH (Medical Subject Headings) terms and keywords. The MeSH terms included "Arnicolide D," "Cancer," "Neoplasms," "Antineoplastic

Agents," and "Mechanisms of Action." The keywords were similarly focused and included phrases such as "anticancer properties," "tumor inhibition," "cell cycle," and "apoptosis." This search identified 287 articles initially.

The inclusion criteria are the following:

- Studies that specifically investigated the effects of Arnicolide D on cancer cells.
- Research published in the last ten years to ensure contemporary relevance.
- Studies that provided precise data on the mechanisms of action of Arnicolide D.
- Articles published in English.

The exclusion criteria are the following:

- Studies that did not focus specifically on Arnicolide D.
- Papers that only explored the general properties of natural compounds in cancer without specific insights into Arnicolide D.
- Research focuses solely on clinical outcomes without addressing underlying mechanisms.
- Non-peer-reviewed articles, abstracts, conference papers, and reviews.

After an initial screening, 102 articles were assessed in full, with 88 ultimately included. Data extraction was performed independently by two researchers, focusing on cancer types, methodologies (in vitro/in vivo), dosages, outcomes, and mechanisms. Discrepancies in data extraction were resolved through discussion and consensus. The data were then synthesized in tables and figures to comprehensively understand the means through which Arnicolide D exerts its anticancer effects. The chemical data has been validated with PubChem (PubChem 2023), and the taxonomy of the plant has been validated with World Flora Online (World Flora Online 2023).

Arnicolide D: a brief overview

Plant sources and description

Arnicolide D is a sesquiterpene lactone in *Centipeda minima*, *Arnica montana*, and *Arnica acaulis* plant sources. *Centipeda minima* (*C. minima*) is a herbaceous plant grown in the eastern tropical region of Asia (Ma and Clemants 2006). The rootlets of the *C. minima* are pale yellow and thin, while the herb itself is yellowish, delicate, and easily removable. Leaves are crumpled with serrated rim edges, appearing brown or green, and the capitulum is a combination of yellowish-brown. The odor is pungent and aromatic with a bitter taste, and it is cultivated in several countries, including Thailand, Russia, the Philippines, Papua New Guinea, Japan, Indonesia, India, and China (Tan et al. 2022). The entire dried plant of *C. minima* extracts Arnicolide D (Wu et al. 1991). *Arnica montana* is available in Europe, Siberia, Mexico, Northwest America, and Canada. It is a yellow flowering plant that grows up to 2 feet tall (Deutsch and Anderson 2008) and contains 0.04% sesquiterpene lactones (Bhardwaj and Misra 2018). Flowers constitute different molecules, such as phenol-carbonic acids, essential oils, acrylic acids, phenyl, hydroxy coumarins, flavonoids, acetylenes, and sesquiterpenes (Kriplani et al. 2017). The plant seeds are composed of flavonoids and phenolic acids (Gawlik-Dziki et al. 2009).

Chemical structure and properties

Arnicolide D, also known as [(1S, 3aR, 5R, 5aR, 8aR, 9S, 9aR)-1,5,8a-trimethyl-2,8-dioxo- 3a,4,5,5a,9,9a-hexahydro-1H-azuleno[6,5-b]furan-9-yl] 2-methylprop-2-enoate (Fig. 1), has the same skeleton as that of medicinally active sesquiterpenoids namely Brevilin A (Lee et al. 2020b) and Arnicolide C (Liu et al. 2019a). Arnicolide D, with the molecular formula C19H24O5, is soluble in acetone, chloroform, acetone, dichloromethane, and dimethyl sulfoxide. Structurally, Sesquiterpene lactones contain one or more lactone rings and three isoprene units and exhibit potent anti-cancer effects (Huang et al. 2014; Su et al. 2020).

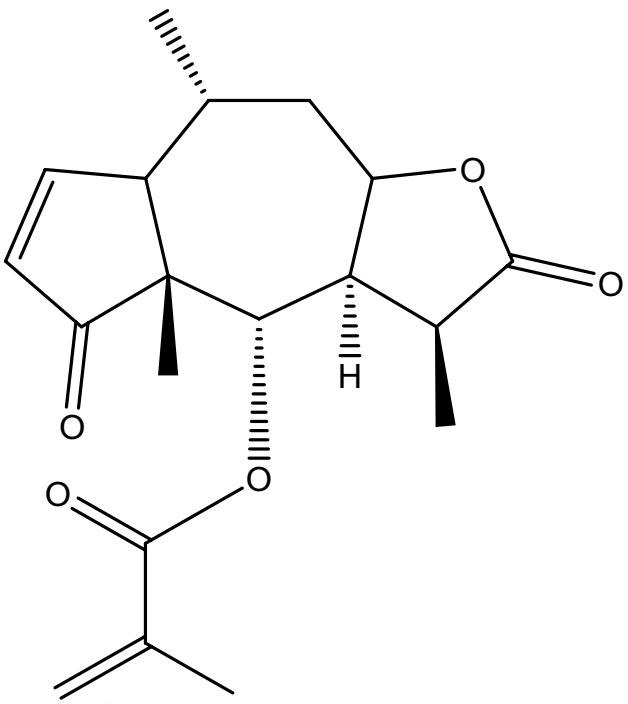

Fig. 1 Structure of Arnicolide D

Chemical structure–activity relationships: critical determinants of Arnicolide D's cytotoxic activity

The structural features of Sesquiterpene lactones are very relevant to biological activity, especially anticancer mechanisms. The primary cytotoxicity mechanism exhibited by the sesquiterpenoids is mainly due to the presence of an alpha-methylene-γ-lactone group, which primarily contributes to the primary biological activity. It involves the formation of irreversible Michael adduct with the nucleophilic center of amino acid residues present in proteins, causing variations in protein structure (Zimmermann et al. 2014). Based on the structure–activity studies of sesquiterpenoids, the primary active site responsible for the cytotoxic antitumor activity is the presence of α, β – unsaturated group (O = C–C-CH_2-) in lactone (Lee et al. 1977). Sesquiterpene lactones bind covalently to sulfhydryl groups and other functional groups of proteins. The binding mechanism can be explained by the Michael-type addition to the electrophilic centre of α, β -unsaturated carbonyl groups (Fig. 2) (Beekman et al. 1997) 36]. The extent of sesquiterpene lactones activity also depends on the number of α, β -unsaturated carbonyl groups present [36]; so, the presence of double bonds in the cyclopentane ring, α, β -unsaturated carbonyl groups and hydroxyl groups are the activity determining components of sesquilactones (Scotti et al. 2007). Also, in the absence of a hydroxyl group, Arnicolide D (Fig. 1) shows significant cytotoxic activities. Based on this observation, we can conclude that an unsaturated group in the cyclopentane ring and the alkene conjugation with the carbonyl group contribute to the antitumor activity. Thus, the presence of lactone ring, α, β -unsaturated carbonyl groups, and cyclopentene ring present in Arnicolide D plays a significant role in determining the drug potency. The literature study shows that the presence of the olefin or carbonyl (C = O) group of the enone moiety is fundamental in exhibiting cytotoxicity. The present compound exhibits all the structural features to show significant activity against cancer cell lines.

Arnicolide's semi-synthetic derivatives and therapeutic potential

Reported literature proved that Arnicolide D is a sesquiterpene lactone and can be used as an anticancer drug. As sesquiterpenoids are biologically active and extensively used as effective precursors or chemical scaffolds for the design and synthesis of anticancer drugs. Current research on anticancer drugs derived from Arnicolide D lacks descriptive findings regarding its novel derivatives, and such outcomes have not yet been reported in the literature. It is expected that Arnicolide D will receive ample consideration as a powerful scaffold in designing schemes for the anticancer drug discovery process. Currently, significant efforts are required for the systematic identification of new derivatives, along with proper structure–activity relationship studies. These efforts are believed to enhance the likelihood of gaining advanced insights in the field of anticancer drugs. Based on [38] evaluations of anticancer potential and structure–activity relationship studies conducted [38], the α, β – unsaturated group is important in attaining cytotoxicity. Also, it is reported that the derivatives of the compounds with *alpha*-position substituents at the γ-lactone ring will show enhanced cytotoxic activity in the cancer cell lines. We must highlight these structural concerns on Arnicolide D and design new derivatives. The existing literature (Huang et al. 2014; Liu et al. 2019a; Zhu et al. 2019a; Qu et al. 2020; Wen et al. 2022) confirms that Arnicolide D possesses the potential to be developed as an anticancer drug. This anticancer potential is rooted in sesquiterpenoids being biologically active and extensively employed as precursors or scaffolds in synthesizing anticancer drugs. Despite this, current research on anticancer drugs derived from Arnicolide D is notably sparse, particularly regarding descriptive findings of its novel derivatives, a gap yet to be filled in scholarly literature (Yao et al. 2022b). It is anticipated that Arnicolide D will receive considerable attention as an effective scaffold in the design of new anticancer drug discovery initiatives (Abu-Izneid et al. 2020). There is a critical need for dedicated efforts to systematically identify new Arnicolide D derivatives

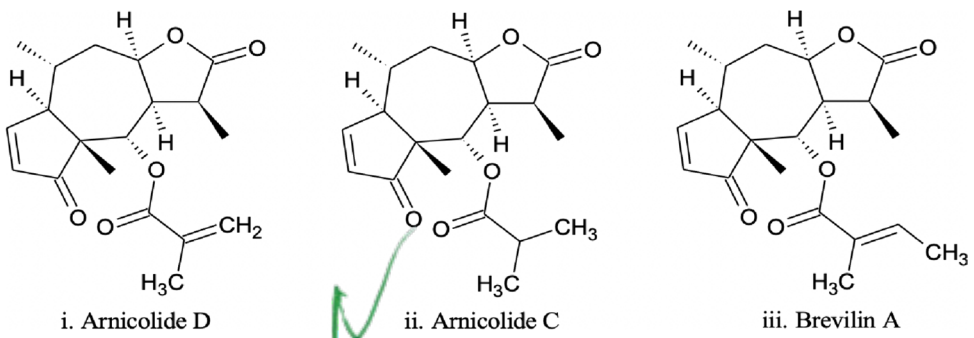

Fig. 2 Chemical structure of sesquiterpene lactones

and comprehensive structure–activity relationship (SAR) studies (Scotti et al. 2007). These endeavors are expected to enhance our understanding and capabilities in anticancer drug development. Specifically, studies have shown that the α, β-unsaturated group in Arnicolide D is pivotal in achieving cytotoxicity (Liu et al. 2024). Furthermore, it has been reported that derivatives with alpha-position substituents on the γ-lactone ring demonstrate increased cytotoxic activity in cancer cell lines (Sülsen et al. 2021). This underscores the importance of focusing on these structural elements of Arnicolide D and innovating new derivatives that exploit these characteristics. Other studies of Arnicolide D have led to the exploration of its semi-synthetic derivatives. These derivatives are engineered to enhance the molecule's therapeutic efficacy by leveraging the biologically active scaffold of Arnicolide D. Modifications primarily targeted the α, β-unsaturated group and the γ-lactone ring, which are pivotal for cytotoxic activity against cancer cell lines. One notable derivative introduced a hydroxyl group at the alpha position of the γ-lactone ring, resulting in significantly increased cytotoxicity against a panel of cancer cell lines, including breast and colon cancer models (Liu et al. 2019b). This modification was shown to enhance the derivative's ability to induce apoptosis through the mitochondrial pathway, as evidenced by increased Bax/Bcl-2 ratio and caspase activation (Liu et al. 2019b). Further, SAR studies have elucidated the fundamental role of the α, β-unsaturated carbonyl group in the lactone ring for binding to cellular nucleophiles, such as cysteine residues in proteins, thereby disrupting cancer cell signaling pathways(Jackson et al. 2017). These insights underscore the significance of structural modifications in enhancing the anticancer potential of Arnicolide D derivatives. Comparative studies highlight the enhanced efficacy of these derivatives over the parent compound. For instance, another semi-synthetic derivatives with an added methyl group at the beta position of the lactone ring, exhibited a lower IC50 value compared to Arnicolide D in nasopharyngeal carcinoma cells, indicating a stronger antiproliferative effect (Liu et al. 2019b; Yao et al. 2022a). The clinical potential of these derivatives is currently under investigation, with preliminary pharmacokinetic studies indicating improved bioavailability and reduced toxicity, suggesting a promising outlook for clinical applications. The exploration of Arnicolide D's semi-synthetic derivatives represents a promising avenue in the quest for novel anticancer agents (Yao et al. 2022a). Continued efforts are required to systematically identify and characterize these derivatives, with a focus on elucidating their mechanisms of action and optimizing their pharmacological profiles; the transition from preclinical models to clinical trials will be critical in assessing the therapeutic viability of these compounds in human cancer treatment (Yao et al. 2022a).

Traditional uses and modern applications

Different parts of *C. minima* are used for various therapeutic purposes worldwide in traditional practices. Arial parts of the plant, stem, leaves, and flowers are used in traditional Chinese medicine to treat ailments including headaches, piles, malaria, cold, conjunctivitis, etc. To treat ophthalmia, the plant was reported to be used in rural Malaysia as a snuff, in Taiwan, the decoction of the plant was reported to be used for rickets, sores, and digestive disorders. Squeezed leaves are inhaled in the Philippines to provoke sneezing to clear the nostrils (Soetardjo et al. 2007). The plant treats coughs, sinus infections, headaches, and colds in Nepal. There are records of the plant used for treating colds, allergies, and asthma in other Southeast Asian parts (Taylor and Towers 1998). In Chinese medicine, the earliest plant forms are seen in Materia Medica for Dietotherapy, written by Meng Shen in the Tang Dynasty of China in about the first century CE. Medical scientist Li Chan, who lived during the Ming Dynasty, claimed the use of the plant in abdominal mass syndrome, which is now translated as tumor (Yao et al. 2022b). In Ayurveda, various ancient texts mention different uses for this herb in different formulations. Charaka Samhita lists Agruvadi taila (oil formulation) used to treat fever, Twagadi tail, and Pradhman Nasya (snuff powder) used as a snuff for various head diseases. Kanakshiri taila is used in treating skin diseases and has *C. minima* as one of the essential ingredients. Ashtanga-sangraha, another ancient text, details different uses of the plant in cough, deworming, and nasal catarrh; Ashtanga-hridayam mentions a ghee-based preparation containing *C. minima* to treat cough (Pulcherio et al. 2013). Recent scientific exploration has extended the potential applications of Arnicolide D beyond traditional uses, uncovering its effectiveness in modern medical treatments. A novel formulation is described in a recently filed patent with the United States Patent and Trademark Office and incorporates *C. minima* as the active ingredient. This formulation protects neuronal cells from oxidative stress, thereby inhibiting apoptosis. The patent outlines that the formulation can enhance the expression of antioxidant enzymes, effectively preventing cellular apoptosis induced by oxidative stress (Li et al. 2020a). The application claims that this preparation can prevent, ameliorate, or treat diseases resulting from such cellular mechanisms and is suitable for pharmaceutical or food products. Another patent discusses an extract preparation from *C. minima*, demonstrating efficacy against various influenza strains, including H1N1, H5N1, and H9N2. This invention details a *C. minima* extract product precisely for antiviral purposes (Liu et al. 2024), which is

reported to contain Arnicolide D, C, -O-tiglic acid-11 alpha, 13-dihydro helenalin, among other components. The inventors claim that the efficacy of this preparation surpasses that of standard drugs in the market (Zhang et al. 2019). Furthermore, the invention proposes that the extract of *C. minima* and its constituents can activate bombesin receptor subtype 3 (BRS3), a G-protein coupled receptor with a broad spectrum of functions, including regulating energy homeostasis and feeding patterns in small animals (Xiao and Reitman 2016). This patent focus on the use of Arnicolide D and Centipeda minima extracts for neuroprotection, specifically in protecting neuronal cells from oxidative stress-induced apoptosis, and for their efficacy against various strains of influenza, including H1N1, H5N1, and H9N2. These applications underscore the broad therapeutic scope of Arnicolide D, extending its potential utility to areas such as neurology and virology, in addition to its noted anticancer properties in preclinical studies.

Pharmacokinetics and bioavailability

Limited data is available regarding the pharmacokinetics of compounds found in *C. minima*. However, comprehensive, particularly concerning the toxicity of Arnicolide D, is accessible (see Sect. 5.5). Due to the absence of evaluations on absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties, Kim et al. employed the computational tool Swiss ADME to predict the ADMET role of various phytochemicals present in *C. minima*, including Arnicolide D. According to this tool, Arnicolide D, along with other phytochemicals in *C. minima*, exhibits high gastrointestinal absorption. Interestingly, it does not interact with cytochrome P450 enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4), typically involved in xenobiotic interactions. This suggests that sesquiterpene lactones in *C. minima* may offer favorable advantages for oral administration (Kim et al. 2021). Oral bioavailability is an important pharmacokinetic parameter to assess a drug's ADME in vivo. Drug-likeness, which refers to the structural similarity between herbal ingredients and known drugs, plays a significant role in new drug development. In 2023, Gao et al. conducted a preliminary screening of compounds from the Traditional Chinese Medicine Systems Pharmacy Database and Analysis Platform to identify active ingredients of *C. minima* influencing a specific type of lung cancer, using parameters of oral bioavailability and drug-likeness. Arnicolide D emerged from this study with an oral bioavailability of 85.85% and a drug-likeness of 0.33, indicating good bioavailability and a promising drug. Subsequently, this preliminary screening was validated in vivo using a lung cancer mouse model, confirming the anticancer effect (Gao et al. 2023). Recent advancements in computational methods have significantly contributed to the prediction of ADMET properties, offering insights into the drug's behavior within the body. The application of graph-based signatures, as demonstrated in the pkCSM (Pharmacokinetics by Chemical Substructure Mining approach), has emerged as a robust method for forecasting central ADMET properties fundamental to drug development, showcasing comparable or superior performance to existing methodologies (Pires et al. 2015). Further, the strategic selection of oral bioavailability-enhancing formulations, underscored by computational evaluations and preclinical ADMET process development, underlines the critical role of lipophilicity, physicochemical properties, and the biopharmaceutical classification system in the optimization of lead compounds (Komura et al. 2023; Niazi and Mariam 2023). This is complemented by the utilization of Quantitative Structure–Activity Relationship (QSAR) models and in silico ADMET predictions (Daoui et al. 2021), which unravel the structural features and pharmacokinetic properties influencing Arnicolide D's anticancer activity, thereby facilitating the development of derivatives with improved efficacy(Olasupo et al. 2020). The advent of SwissADME, a freely accessible web tool, has further revolutionized the evaluation of pharmacokinetics, drug-likeness, and medicinal chemistry friendliness, providing an integrated platform to rapidly assess key parameters for a collection of molecules. This tool aids in the prediction of physicochemical properties, pharmacokinetics, and potential toxicity, thereby supporting drug discovery endeavors (Daina et al. 2017; Tran et al. 2023). To ensure the reliability of these computational predictions, it is necessary to validate them through in vivo studies (Van Calster et al. 2023). The exploration of Arnicolide D's pharmacokinetic profile in animal models can provide concrete evidence of its absorption, distribution, metabolism, and excretion, thereby corroborating the computational forecasts and paving the way for clinical applications. The integration of computational predictions with empirical data is important for advancing our understanding of Arnicolide D's pharmacological potential and optimizing its therapeutic efficacy. Arnicolide D presents promising therapeutic potential, but its oral bioavailability faces several challenges. The compound's inherent physicochemical properties, such as solubility and stability in the gastrointestinal tract, could limit its effective absorption upon oral administration. Also, the extensive first-pass metabolism in the liver may significantly reduce the systemic availability of Arnicolide D, necessitating higher doses for therapeutic efficacy, which could lead to an increased risk of side effects. Another potential limitation is the interaction of Arnicolide D with intestinal transporters and enzymes, which can further complicate its absorption profile. Additionally, the compound's ability to cross biological membranes efficiently remains to be fully elucidated, posing a challenge to achieving adequate plasma

concentrations. Future research should focus on overcoming these limitations through the development of novel delivery systems, such as nanoparticles, liposomes, or microemulsions, which could enhance the solubility, stability, and absorption of Arnicolide D. Investigating prodrug strategies that improve its pharmacokinetic properties could also be beneficial. Furthermore, comprehensive in vivo studies are essential to validate computational ADMET predictions and to understand the metabolism and distribution patterns of Arnicolide D in the body.

Arnicolide D's cellular impact in cancer: inhibiting tumor growth, apoptosis induction and targeting cellular survival mechanisms

The main hallmarks of cancer are widely studied and complex and are a very passionate area to explore, and there are many reviews elsewhere (Fig. 3) (Cragg et al. 2009; Nenclares and Harrington 2020). In genetic alterations leading to neoplasia, three important biologic pathways that normally regulate cell growth and tissue homeostasis are affected: cell cycle, apoptosis, and differentiation. These pathways are intimately integrated, so disrupting one pathway can have significant consequences in another (Corn and El-Deiry 2002). In this sense, one defining feature of cancer is the uncontrolled cellular proliferation resulting from disturbances in numerous regulators orchestrating the correct progression through the cell cycle. Notably, many human cancers exhibit dysregulated control over the progression of the G1 phase, influencing the delicate balance between proliferation and quiescence (Corn and El-Deiry 2002). Another strategy cancer cells employ involves evading apoptosis, either by ignoring signals from trimeric death ligands (extrinsic pathways) or altering the equilibrium between pro- and anti-survival molecules; this avoidance is pivotal for cancer cells to persist despite DNA damage (Nenclares and Harrington 2020). Moreover, cancer cells exhibit aberrations in antigrowth signaling pathways, disrupting the regulation of cell cycle progression. Typically, anti-growth signals function by inducing cellular quiescence or terminal differentiation. However, this regulatory mechanism in cancer cells is compromised, leading to continued re-entry into the cell cycle (Nenclares and Harrington 2020). As mentioned earlier, ethanolic extract from *C. minima* has been used for centuries in traditional Chinese medicine due to the promising results observed in different ailments, including cancer. Ethanol extract of *C. minima* significantly inhibited triple-negative breast cancer (TNBC) metastasis by the inhibition of different signaling pathways, including PI3K/AKT/mTOR, nuclear factor kappa B (NF-κB), and signal transducer and activator of transcription 3 (STAT3) signaling pathways (Lee et al. 2020a). Another study suppressed the proliferation and induced apoptosis by inhibiting the PI3K/AKT/mTOR signaling pathway in nasopharyngeal carcinoma (NPC) cells (Guo et al. 2015). Interestingly, in 2023, Gao et al. showed that the combination of the ethanolic extract from *C. minima* with the widely used drug cisplatin (first-line treatment of lung cancer) has a synergistic effect by enhancing apoptosis of cancerous cells through the reduction of the anti-apoptotic Bcl2 expression; this result was also demonstrated in non-small cell lung cancer xenograft (Gao et al. 2023). Since Arnicolide D is one of the primary compounds present in *C. minima*, with an average content of 0.58 mg/g in methanol extract (Chan et al. 2019), it would be interesting to expand the investigation of Arnicolide D and cancer. Next, we will mention the available evidence about Arnicolide D and the mechanism of action to have its anticancer effect.

Cell proliferation and cell cycle regulation

In normal conditions, cells use glucose for energy, generating 38 ATP molecules per glucose through glycolysis and oxidative phosphorylation. In contrast, cancer cells switch to glycolysis to produce lactate even in oxygenated conditions (Warburg effect), producing only 2 ATP per glucose (Liberti and Locasale 2016). However, this metabolic shift benefits cancer cells by increasing necessary substrates for growth and division (Nenclares and Harrington 2020). The cell cycle regulation is tightly controlled by many regulatory mechanisms that permit or restrain its projection (Gali-Muhtasib and Bakkar 2002), while in cancer, this regulation is profoundly affected by various molecular alterations (Golias et al. 2004). Firstly, the leading families of regulatory proteins that play key roles in controlling cell-cycle progression and that comprise the basic regulatory machinery responsible for catalyzing cell cycle transition and checkpoint traversation are the cyclins, cyclin-dependent kinases (Cdks), their substrate proteins, the Cdk inhibitors (CKI), and retinoblastoma protein (Golias et al. 2004; Nenclares and Harrington 2020). When the antigrowth signaling pathways fail, cancer might appear. The loss of members of the CDK inhibitor family and overexpression of cyclins and CDKs occur in many types of tumors (Nenclares and Harrington 2020). Additionally, another critical aspect of cell cycle regulation in cancer involves proto-oncogenes. Proto-oncogenes are tightly regulated to avoid uncontrolled cell growth and changes in their behavior or protein structure, which can transform them into oncogenes, driving uncontrolled cell multiplication and tumor progression; many proto-oncogenes are involved in signaling pathways that directly regulate cell cycle entry and exit; exerting an important role in the regulation of cell proliferation (Kontomanolis et al. 2020). The molecular action of the antitumor mechanism of

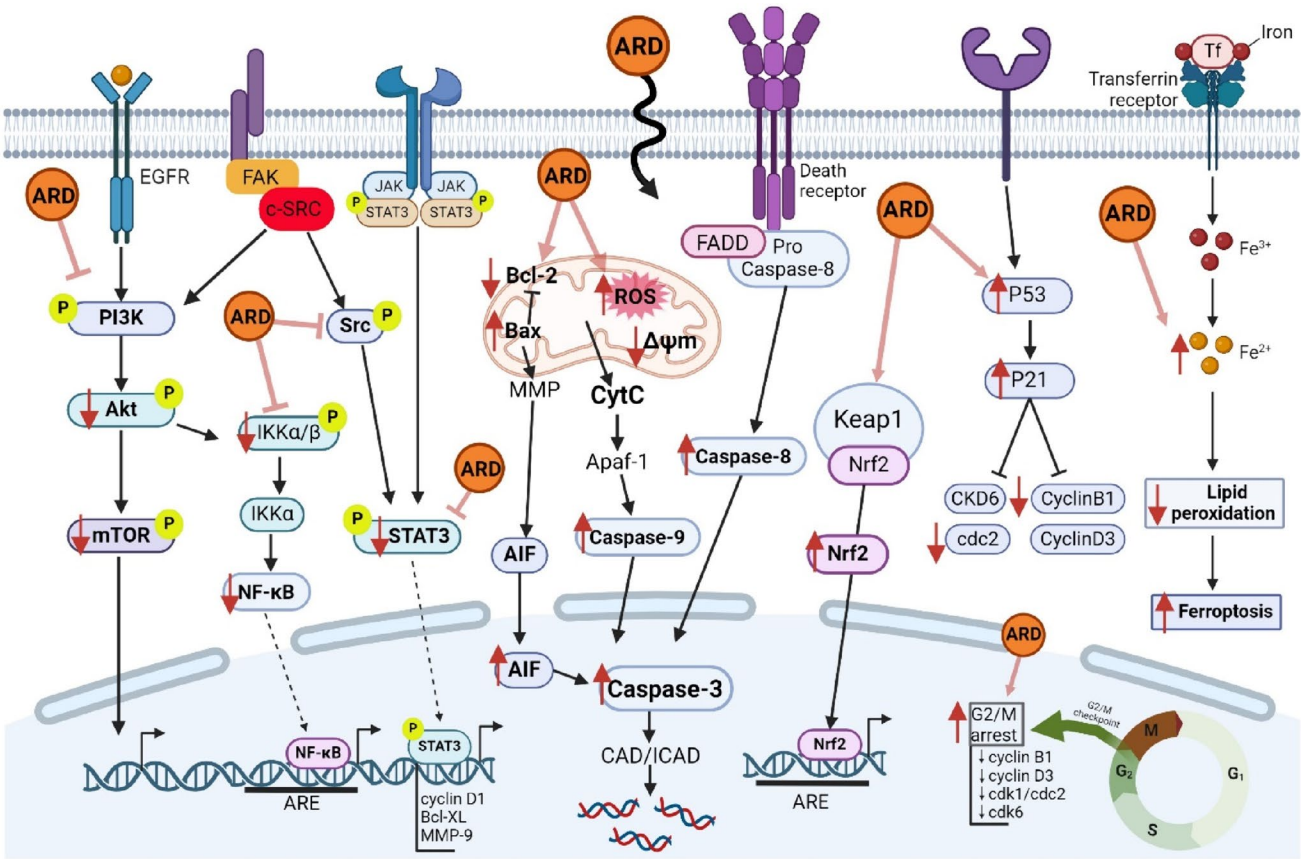

Fig. 3 Mechanisms of antitumor action of Arnicolide D. The figure depicts the impact on cellular signaling pathways in cancer cells, highlighting its role in apoptosis, cell cycle arrest, and ferroptosis. ARD induces apoptosis through mitochondrial disruption, evident by Bax activation, Cytochrome C release, and subsequent caspase cascade activation. It promotes cell cycle arrest by stabilizing P53, which enhances P21 expression, leading to downregulation of cyclins and cyclin-dependent kinases. ARD also initiates ferroptosis via iron accumulation and lipid peroxidation. Concurrently, ARD influences other critical pathways, such as PI3K/Akt/mTOR and JAK/STAT3, which are involved in cell survival and proliferation, and the Nrf2 pathway for oxidative stress response, portraying a comprehensive anti-tumor strategy. AIF (Apoptosis-inducing factor); Akt (Protein kinase B); Apaf-1 (Apoptotic protease activating factor 1); ARE (Antioxidant response element); ARD (Arnicolide D); Bax (Bcl-2-associated X protein); Bcl-2 (B-cell lymphoma 2); Bcl-XL (B-cell lymphoma-extra large); CAD/ICAD (Caspase-activated DNase/inhibitor of CAD); Caspase-3, -8, -9 (Cysteine-aspartic proteases); Cdc2 (Cell division cycle protein 2); Cdk6 (Cyclin-dependent kinase 6); CytC (Cytochrome c); EGFR (Epidermal growth factor receptor); FADD (Fas-associated protein with death domain); FAK (Focal adhesion kinase); Fe2+(Ferrous ion); Fe3+(Ferric ion); IKKα/β (IκB kinase α/β); JAK (Janus kinase); Keap1 (Kelch-like ECH-associated protein 1); MMP (Mitochondrial membrane potential); mTOR (Mammalian target of rapamycin); NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells); Nrf2 (Nuclear factor erythroid 2–related factor 2); P21 (Cyclin-dependent kinase inhibitor 1); P53 (Tumor protein p53); PI3K (Phosphoinositide 3-kinase); ROS (Reactive oxygen species); Src (Proto-oncogene tyrosine-protein kinase Src); STAT3 (Signal transducer and activator of transcription 3); Tf (Transferrin)

Arnicolide D can be explained based on cell cycle regulation and apoptosis induction along with the presence of essential structural components α and β-unsaturated carbonyl moieties (Wu et al. 2012). Liu et al. have documented the anti-nasopharyngeal carcinoma (NPC) effects of Arnicolide D in a range of concentrations from 0 to 50 μM on the NPC cell lines CNE-1, CNE-2, SUNE-1, HONE1, and C666-1. The considerable cell growth inhibition explained the shown effect, cell cycle arrest in the G2/M phase, and suppression of the PI3K/AKT/mTOR signaling pathway after 24 h and 48 h, dependent on time and concentration. Also, there was a decrease in expression levels of proteins cyclin D3, cyclin B1, cdk1/cdc2, and cdk6 (Liu et al. 2019a). Arnicolide D is also effective against Triple Negative Breast Cancer (TNBC) in the cell lines MDA-MB-231 and MDA-MB-468. The proportion of MDA-MB-231 cells in the G2/M phase significantly increased after 5 μM of treatment for 24 and 48 h. In contrast, interestingly, higher doses of Arnicolide D exhibited weaker inhibition of the cell cycle, suggesting that the antiproliferative effect may be through cell cycle arrest combined with other mechanisms at higher doses (Qu et al. 2020). Moreover, 4 μM of Arnicolide D exerted

anti-melanoma effects by G2/M cell cycle arrest, as well as downregulating the G2/M checkpoint proteins, such as cdc2, cyclin B1 in both A375 and B16F10 melanoma cells (Zhu et al. 2019a).

Induction of apoptosis

Cancer cells evade apoptosis through an ability to ignore signals sent through the extrinsic pathway or by resetting the balance of intracellular pro- and anti-survival molecules towards inhibition of apoptosis. Cancer cells can sustain DNA damage by circumventing apoptosis without causing cell death (unless the damaged gene is necessary for cell survival) (Qian et al. 2022). Huang et al. demonstrated that Arnicolide D has anticancer activity by inhibiting the signaling pathways NF-κB, PI3K/Akt/mTOR, and STAT3. Notably, in the human colon HT-29 cell line, a concentration of 3 μM was able to inhibit the translocation of NF-κB, and consequently, induced apoptosis (Huang et al. 2014). Furthermore, Arnicolide D suppressed NF-κB signaling by inhibiting both IKKα/β phosphorylation and IκBα degradation in A375 and B16F10 melanoma cells (Zhu et al. 2019b). Moreover, Arnicolide D could decrease the phosphorylation levels of PI3K, Akt, mTOR, and STAT3, thereby resulting in the apoptosis of CNE-2 and triple-negative breast cancer (TNBC) cells (Liu et al. 2019a; Qu et al. 2020).

Arnicolide D also significantly increased the proportion of apoptotic cells at doses of 10 and 20 μM in the TNBC cell lines MDA-MB-231 and MDA-MB-468; distinct chromatin condensation and formation of apoptotic bodies was observed after 24 h treatment of Arnicolide D (Qu et al. 2020). Anti-nasopharyngeal carcinoma activity through mitochondrial apoptosis pathway occurred in a dose/time dependent manner (0–50 μM during 24 or 48 h) on CNE-2 and NPC cells, inducing characteristic apoptotic morphological changes in cells (increased chromatin condensation, apoptotic bodies, shrinkage, and nuclear degradation), and increase in early/late apoptosis. After treatment with 1.25 to 20 μM of Arnicolide D during 24 or 48 h, pro-apoptotic activated proteins (PARP, caspase-9, and Bax) were increased, while anti-apoptotic proteins were decreased (Bcl-2) (Liu et al. 2019a). In another study, Arnicolide D decreased mitochondrial membrane potential in breast cancer cells, thereby inhibiting cell viability and increasing lactate dehydrogenase (LDH) release. Also, treatment activated the classical apoptosis pathway to induce cell apoptosis; it significantly promoted PARP-1 expression, enhanced the nuclear translocation of apoptosis-inducing factor (AIF), and reduced the expression of AIF in mitochondria. Importantly, in this work, they confirmed Arnicolide D´s activity by silencing the apoptosis and PARP inhibitor with siRNAs and significantly attenuated the inhibitory effect of Arnicolide D, indicating that this can inhibit MDA-MB-231 and MDA-MB-468 breast cancer cell lines growth by inducing apoptosis (Wen et al. 2022).

Inhibition of survival pathways

mTOR, targeted by sirolimus or rapamycin, is a conserved serine/threonine protein kinase regulating fundamental cellular processes (Saxton and Sabatini 2017), which integrates multiple signals from extracellular and intracellular signaling pathways (responsible for the regulation of many fundamental cells and biological processes), it phosphorylates targets involved in cell growth, autophagy, metabolism, and cancer processes; dysregulation in mTOR signaling is linked to various cancers (Bouyahya et al. 2022; Cheng et al. 2023). mTOR, MAPK, and NF-kB are critical pathways in cancer formation, with mTOR playing an important role in apoptosis, cell cycle, and proliferation. mTOR´s diverse functions in carcinogenesis include glycolysis activation and regulation through parallel pathways and feedback loops. Phosphorylation of mTOR after Akt activation is important for controlling cell processes, and mutations in TSC2, PTEN, and other ways contribute to mTOR dysregulation in various cancers. By changing the cell morphologies, Arnicolide D inhibited the PI3K/AKT/mTOR and STAT3 signaling pathways. Fluorescent (DAPI) studies showed the presence of sub-lobular cell nuclei with fragmented outlines. These reports on the anticancer mechanism provide strong evidence for Arnicolide D as an anti-nasopharyngeal carcinoma compound for further research (Liu et al. 2019a). In breast cancer, mTOR signaling activation is associated with increased tumor progression and reduced patient survivability (Bouyahya et al. 2022). In the TNBC cell lines MDA-MB-231 and MDA-MB-468, Arnicolide D significantly inhibited the expression of Akt and mTOR and their phosphorylated forms. Since the PI3K/Akt/mTOR signaling pathway plays a significant role in controlling cell growth, proliferation, and metastasis, that pathway has been identified as an important potential therapeutic target in breast cancer (Qu et al. 2020). Reactive oxygen species (ROS) can influence cell survival by modulating the response of nuclear factor-kB (NF-kB) proteins. NF-kB is a family of transcription factors that regulate the expression of numerous genes involved in cell growth, differentiation, development, and apoptosis (Morgan and Liu 2011). Additionally, several sesquiterpenoids have been reported as potent inhibitors of NF-kB signaling (Fraga 2002). Huang et al. determined the effects of 3 μM Arnicolide D in HT-29 colon cancer cells. They found that the ROS levels were significantly increased within 30 min and maintained until 4 h, and the level of NF-kB in the nucleus of HT-29 cells was decreased after incubation for 24 h. These results suggest that the increase in ROS and inhibition of NF-kB are involved in the mechanism of cell cycle arrest and apoptosis;

the mechanism of cytotoxic activity was associated with the G1 arrest, increased ROS, and down-regulation of NF-kB levels (Huang et al. 2014).

Impact on tumor growth and metastasis

In the context of cancer, a tumor´s ability to progress is closely tied to its capacity to secure adequate blood supply (Nenclares and Harrington 2020). Tumors overcome oxygen and nutrient limitations through angiogenesis, a process regulated by the interplay between pro- and anti-angiogenic factors. The "angiogenic phenotype" of tumors is characterized by overexpression of proangiogenic growth factors like vascular endothelial growth factor (VEGF) and/or suppression of antiangiogenic factors such as angiostatin (Nenclares and Harrington 2020). This dysregulation results in inefficient yet functionally active tumor vascularization (Nagy and Armbruster 2012). Additionally, invasion and metastasis, important stages in tumor progression, involve a series of complex biological processes, from the dissociation of tumor cells to their establishment at a new site, including degradation of the extracellular matrix, directional migration, localized angiogenesis at the metastatic site, and activation of proteins like Src (Nenclares and Harrington 2020). The STAT3 and PI3K/AKT/mTOR signaling pathways are downstream targets of Src. Src, a proto-oncogene tyrosine kinase known as c- Src, belongs to the family of non-receptor tyrosine kinase proteins, specifically within the typical members of Src family kinases (SFKs) (Li et al. 2023). SFKs play key roles in many cellular activities, including cell morphology, motility, proliferation, survival, and vasoconstriction. Src inhibitors are of interest as antitumor agents. Yao et al. revealed that the anti-prostate cancer effect of Arnicolide D (8 µM or 30 µM) that reduced 50% or 90%, respectively, of p-Src expression in PC3 and DU145 cells, is due to the binding interaction with Src, which is also confirmed by the molecular docking studies. These studies reveal that Arnicolide D can promote Src degradation in prostate cancer cells by shortening the Src protein half-life. Also, the reports reveal that MET341 residues are the essential binding sites of Arnicolide D and Src based on molecular docking (Yao et al. 2022b). STAT3 is an important transcription factor, and its overexpression and constitutive activation play an important role in the progression, proliferation, and metastasis of breast cancer. Qu et al. reported the in vitro (MDA-MB-231 and MDA-MB-468) and in vivo (MDA-MB-231 xenograft mouse model) inhibitory effect of Arnicolide D against TNBC cell line growth. The mechanism of inhibitory effects was associated with the Akt/mTOR and STAT3 signaling. Very interestingly, this study demonstrated a suppression of cell migration with Arnicolide FD (2.5–10 µM) after 24 and 48 h of treatment, in vitro; also, a decrease in the tumor size was confirmed in vivo after oral administration of 25 or 50 mg/kg of Arnicolide D, which led to a reduction of tumor weights by 24.7% or 41.0%, respectively, after 22 days and without appreciable side effects. Here, Arnicolide D significantly downregulated the expression of STAT3 and p-STAT3 in a dose-dependent manner in MDA-MB-231 cells (Qu et al. 2020) (Fig. 4). Prostate cancer was predicted to be the most sensitive cancer type to Arnicolide D, which may directly bind to Src kinase based on molecular docking, inhibiting the phosphorylation of Src and STAT3. These studies strongly indicate that Arnicolide D could be developed as a promising anticancer agent. Notably, Arnicolide D did not cause clinical signs of toxicity in mice and showed no hepatotoxicity (Zhu et al. 2019a), suggesting its safety for in vivo treatment.

Cytotoxic action and reactive oxygen species (ROS)

Reactive oxygen species (ROS) are essential in cellular damage that ends in cancer. ROS are generated by multiple endogenous and exogenous factors, which can lead to biological consequences. In normal conditions, ROS acts as an intracellular second messenger. Moderate ROS levels benefit cancer cells by promoting increased cancer metabolism and growth signaling and inhibiting antioxidants, which contribute to oncogenesis. High ROS levels of mitochondrial ROS can release cytochrome c into the cytosol from the mitochondrial intermembrane space and/or activate transmembrane death receptors, beginning the apoptosis cascade signaling pathway (Nakamura and Takada 2021). The nuclear factor erythroid 2-related factor 2 (Nrf2)-Kelch-like ECH-associated protein 1 (KEAP1) regulatory pathway is a pivotal regulator in cellular and tissue protection from different internal and external stresses, including oxidative stress (Panieri et al. 2020). Arnicolide D has been shown to suppress the oxidative stress response by activating the ERK/Nrf2 signaling pathway, being Arnicolide D one of the two active compounds responsible for the activation of the Nrf2 signaling pathway and inhibition of ROS production (Wang et al. 2019). Since elevated levels of ROS provoke cellular death, this high level of cytotoxicity is bad for tumor progression. Recently, Wen et al., in 2022, showed an exciting activity of Arnicolide D to combat breast cancer; here, they showed that Arnicolide D increases ROS and cytotoxicity to induce apoptosis, ferroptosis (iron-dependent cell death with lipid peroxidation and membrane damage) and parthanatos (cell death via PARP-1 hyperactivation, causing PAR accumulation and NAD + depletion). In this case, Arnicolide D promoted ROS production, reduced glutathione peroxidase 4, lipid peroxidation, and hyperactivation of poly (ADP-ribose) polymerase-1 (PARP-1), and all together underscore the significant anti-tumor potential of Arnicolide D (Wen et al. 2022). Also, Arnicolide D treatment in the colon cancer cell line HT-29 amplified intracellular ROS levels and

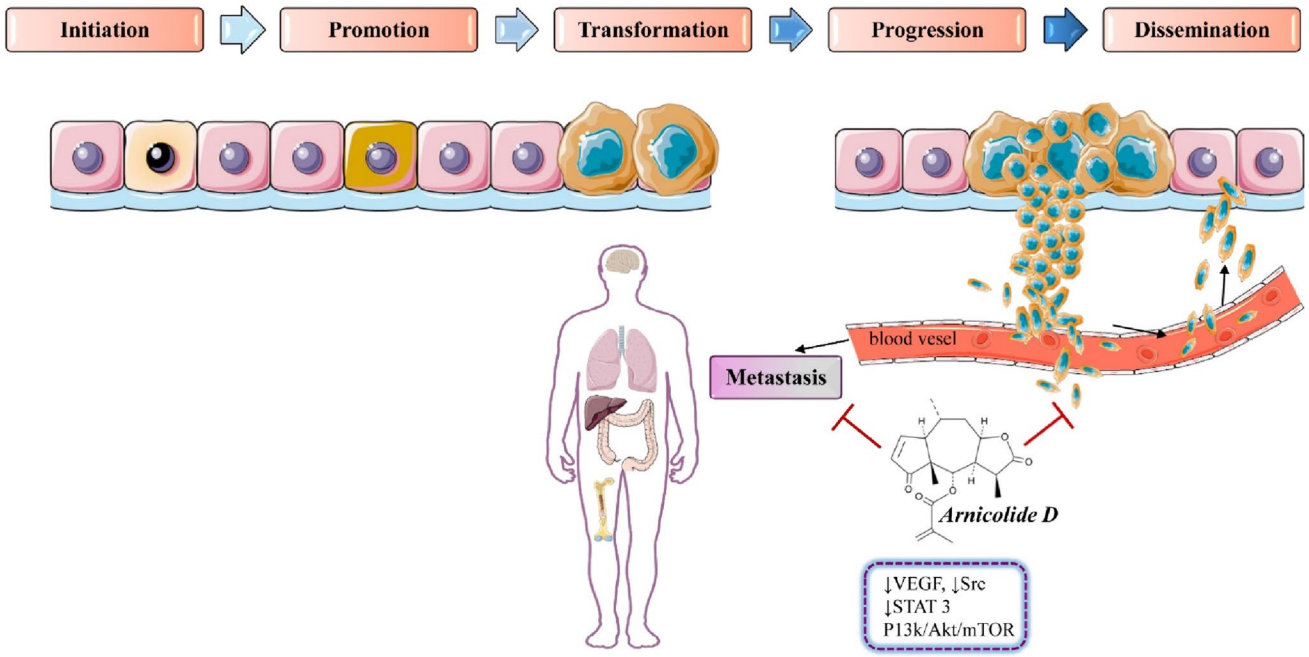

Fig. 4 Inhibitory impact of Arnicolide D on cancer progression and metastasis. The figure depicts cancer progression from initiation to metastasis and the intervention points of Arnicolide D. It highlights the compound's downregulation of VEGF, Src, STAT3, and PI3K/Akt/mTOR, important for tumor growth and spread. Abbreviations: VEGF, vascular endothelial growth factor; Src, protein kinase; STAT3, transcription activator; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; mTOR, target of rapamycin

reduced the levels of NF-κB, subsequently causing apoptosis and G1-phase cell cycle arrest (Huang et al. 2014). In this sense, some researchers have reported the inhibitory concentration of 50% (IC_{50}) in different cancer cell lines, showing a reduction in cellular viability in a dose- and time-dependent manner (see Table 1).

Arnicolide D: multi-pathway modulation and anticancer efficacy in preclinical cancer models

In a recent study, Zhu et al. (Zhu et al. 2019a) evaluated the anticancer activity of Arnicolide D in melanoma cells to explore its therapeutic characteristics in two in vitro models: A375, a human cell line that carries mutant genes B-RAF and CDKN2, both related to skin damage (Avram et al. 2017); and B16F10, a murine cell line which exhibits a tremendous metastatic capacity (Shen et al. 2023). In an in vivo model utilizing C57/BL6 mice with B16F10 allografts in their backs (Li et al. 2017), this mice strain may have been selected because of its favorable immunological properties, particularly its cell-mediated and natural killer (NK) response (Song and Hwang 2017). Regarding their results about the in vitro models, A375 cells showed a significant decrease in cell viability as early as with the 1 μM exposure up until de 4 μM concentration for 48 h, following a dose-dependent behavior; these cell lines also experimented with a cycle arrest at G2/M-phase checkpoint, as well as an increase in the apoptotic rate when exposed to 2 and 4 μM. Concerning protein level expression, Western blot revealed an increment in the expression of p53, p21, and IκBα proteins while decreasing the expression of CyclinB1, Cdc2, p-IKKα/β, p65, and p-p65 proteins at 4 μM treatment, further analyzed. Meanwhile, the B16F10 line also showed a significant lowering in cell viability from 1 up to 4 μM concentrations done for 48 h, accompanied by an increase in p53, p21, and IκBα protein levels, and a decrease in protein levels of CyclinB1, Cdc2, p-IKKα/β, p65, and p-p65. According to their results, the cell cycle arrest at the G2/M-phase is an important checkpoint before mitosis, where the cell's proteins detect any DNA damage and repair it before starting the next step; this is fundamental for maintaining chromosomal stability (Stark and Taylor, 2024). This arrest can be explained by the increased amount of p53, which inhibits the translation of Cdc2 and cyclin B1 that are involved in the progression from G2 to M phase (Zhu et al. 2019a); p21 also acts as a cell cycle inhibitor by binding to cyclin D/cdk4 and cyclin E/cdk2 complexes during G1/S stage (Brugarolas et al. 1999). The rise in the apoptotic rate can also be elucidated with the upper expression of p53; mutations detected by the protein that cannot be restored cause Bax transactivation, translocating it from the cytoplasm to organelle's membranes, followed by a release

Table 1 Main findings on the biological activity of Arnicolide D

Model	Type of cancer	Cell proliferation	Cell cycle regulation	Apoptosis	Survival pathways	Tumor growth/ metastasis	Cytotoxicity action (μM)/ROS	Tx	References
In vitro CNE-1; CNE-2; SUNE-1; HONE1; C666-1	NPC	↓Cell growth	↓ cyclin D3, cyclin B1, cdk1/cdc2, cdk6 Cell cycle arrest (G2/M phase)	↑ apoptotic morphology ↑ pro-apoptotic proteins (PARP, caspase-9, Bax) ↓ anti-apoptotic proteins (Bcl-2)	↓ PI3K p110α p-PI3K p85 p-Akt, p-mTOR ↓ p-STAT3	N/A	IC_{50} CNE-2: 0.99 at 48 h; 0.83 at 72 h	In vitro 0–50 μM (24, 48, and 72 h)	(Liu et al. 2019a)
In vitro MDA-MB-231; MDA-MB-468 In vivo MDA-MB-231 xenograft	TNBC	N/A	↑ G2/M phase	N/A	↓ Akt/mTOR ↓ STAT3, p-STAT3	↓ cell migration ↓ tumor size	IC_{50} MDA-MB-231 (12.04 at 24 h; 5.211 at 48 h; 3.258 at 72 h) IC_{50} MDA-MB-468 (9.507 at 24 h; 3.405 at 48 h; 2.515 at 72 h)	In vitro: 2.5–20 μM (24, 48 h) In vivo: 25 or 50 mg/kg (oral)	(Qu et al. 2020)
In vitro A375; B16F10 In vivo B16F10 cell allograft	Melanoma cancer	N/A	↓ G2/M checkpoint ↓ cdc2, cyclin B1 ↑ p53, p21	↑ apoptosis	↓ p-IKKα/β ↓ degradation of IκBα ↓ p-NF-κB p65 ↓ p-STAT3	↓ p-Src	N/A	In vitro 4 μM In vivo 4 mg/kg (i.p.)	(Zhu et al. 2019a)
In vitro HT-29	Colon cancer	N/A	↓ G1-phase cell cycle arrest	↑ apoptosis	↓ NF-κB ↓ PI3K/Akt/ mTOR ↓ STAT3	N/A	IC_{50} HT-29 (2.90 at 72 h) ↑ ROS levels	In vitro 3 μM (24 h)	(Huang et al. 2014)
In vitro MDA-MB-231; MDA-MB-468	Breast cancer	↑ ferroptosis (↓ GPX4, ↓ lipid peroxidation) ↑ parthanatos (↑ PARP-1)	N/A	↓ ΔΨm ↑ PARP-1 ↑ nuclear translocation of AIF ↓ AIF in mitochondria	N/A	↓ MMP-2 ↓MMP-9	↓ cell viability ↑ LDH release ↑ ROS	In vitro 1–5 μM (24 h)	(Wen et al. 2022)
In vitro PC3; DU145	Prostate cancer	N/A	N/A	N/A	N/A	↓ p-Src ↓ Src protein half-life	IC_{50} PC3 (26.68 at 24 h; 9.73 at 48 h) IC_{50} DUI45 (88 at 24 h; 47.84 at 48 h)	In vitro 8 μM or 30 μM	(Yao et al. 2022b)

Tx, treatment; *ROS*, reactive oxygen species; *N/A*, not available; *NPC*, nasopharyngeal carcinoma; *TNBC*, triple negative breast cancer; *LDH*, lactate dehydrogenase; *AIF*, apoptosis-inducing factor; *PARP-1*, poly (ADP-ribose) polymerase-1; *GPX4*, glutathione peroxidase 4; *MMP*, matrix metallopeptidase; *ΔΨm*, mitochondrial membrane potential; *i.p.*, intraperitoneal. Symbols: ↑ increase, ↓ decrease

of cytochrome c from mitochondria and activating the caspase -9, -3, -6, and -7 pathway, causing apoptosis (Shen and White 2001). Arnicolide D was shown to inhibit the activation by phosphorylation of IKKα/β, increasing the levels of IκBα and decreasing the p65 and p-p65 ones; the last ones are involved in determining the duration, specificity, and strength of the NF-κB effect (Fig. 5) (Kwon et al. 2016). Overall, Arnicolide D stopped all the IKK/IκBα/NF-κB p65 signaling pathways that are involved in multiple physiological processes, such as inflammation, cell cycle, proliferation, and cell death; their malfunction can be related to human diseases such as arthritis, atherosclerosis and cancer (Mathes et al. 2008; Christian et al. 2016), but the relation between the inhibition of this pathway and the p53 activations needs to be further explored (Zhu et al. 2019a).

For the in vivo results, the tumor's size, volume, and weight were significantly smaller than the control group in a 4 mg/kg dose. Contradictorily, 8 mg/kg doses were like the control group in the above characteristics. The intraperitoneal administration of Arnicolide D did not provoke any side effects, like increased body weight during the experimental period. This differs from the positive control group, where the mice experimented with body weight loss (Zhu et al. 2019a). In another study by Liu et al. (Liu et al. 2019a), Arnicolide D anticancer activity was assessed against five nasopharyngeal carcinoma cell lines, and the results were like those mentioned earlier. Cytotoxicity was achieved in a time- and concentration-dependent manner. Its effects could be seen as soon as 24 and 72 h, with significant inhibitory concentrations from 1.56 to 50 µM. Cell morphology was also evaluated, and it was observed that all cultures exhibited apoptotic changes, such as cell shrinkage, chromatin condensation, formation of apoptotic bodies, and nuclear degradation, also shown in a concentration-dependent manner. About the cell cycle distribution, it was shown that Arnicolide D arrested cells in the G2/M-phase after 24 and 48 h

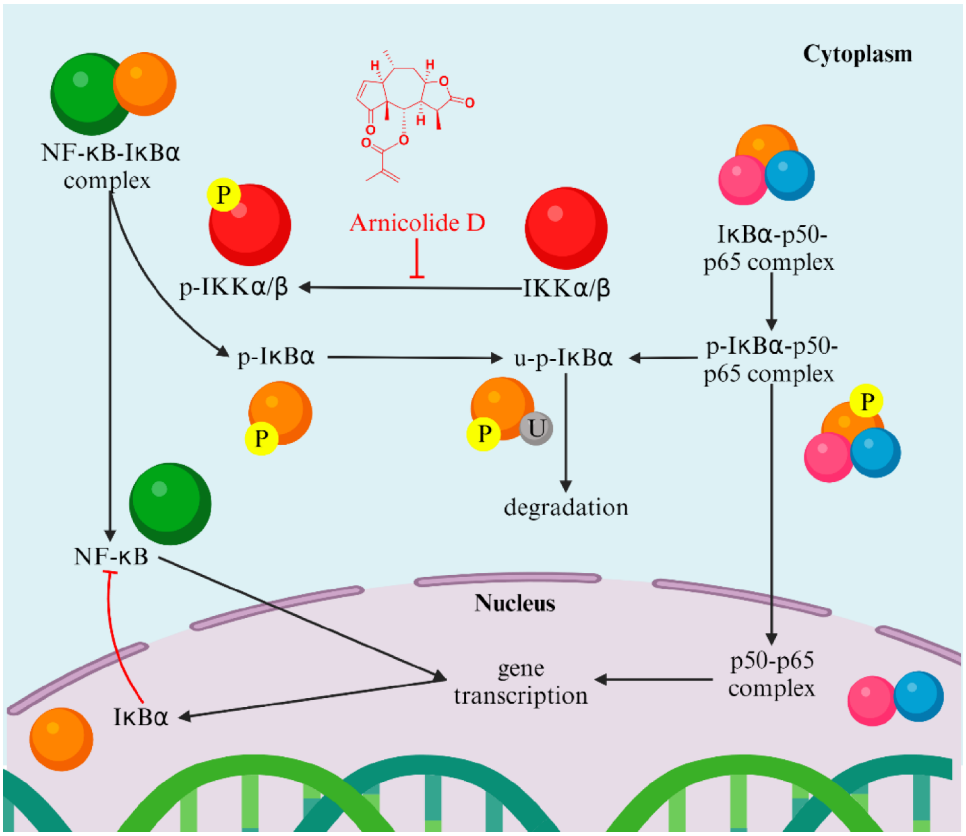

Fig. 5 Arnicolide D mechanism of inhibition of the IKK/IκBα/NF-κB p65 pathway in cancer cells. This figure illustrates the specific inhibitory action of Arnicolide D on the NF-κB signaling pathway within the cytoplasm and nucleus of a cell. Arnicolide D interferes with the phosphorylation of IκBα by IKKα/β, which is a critical step for the activation of NF-κB. Normally, NF-κB is sequestered in the cytoplasm by IκBα. Upon phosphorylation by IKKα/β, IκBα undergoes ubiquitination and subsequent degradation, releasing NF-κB to translocate into the nucleus and initiate gene transcription. However, the presence of Arnicolide D prevents the phosphorylation and degradation of IκBα, thereby inhibiting the release and nuclear translocation of NF-κB. This results in the suppression of NF-κB target genes involved in inflammation, cell proliferation, and survival. IκBα (inhibitor of kappa B alpha); IKKα/β (IκB kinase alpha/beta); NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells); p (phosphorylated); p50/p65 complex (NF-κB dimers consisting of p50 and p65 subunits); u (ubiquitin)

of treatment with concentrations of 1.25, 2.5, 5, 7.5, and 10 µM, also in a concentration and time-dependent manner, this arrest was accompanied by a decrease in cells in G1 stage. Western blot showed that levels of cyclin B1, Cdc2, and cdk6 were augmented in concentrations of 1.25, 2.5, and 5 µM, and then its levels diminished concentration-dependently. Levels of Cyclin D3 lowered from 1.25 to 20 µM exposure treatment. Regarding its pro-apoptotic effects, Arnicolide D induced apoptosis on all studied cell lines, being exposed to concentrations of 1.25, 2.5, 5, 7.5, 10, 20, and 50 µM for 24 and 48 h, exhibiting pro-apoptotic effects in a concentration and time-dependent behavior. Western blot showed increased levels of cleaved PARP, a critical polymerase that is involved in DNA repair, and biochemical and molecular signaling; when it is cleaved by caspases, it is a hallmark of apoptosis; it binds irreversibly to DNA and inhibits its repair (Chaitanya et al. 2010); increased levels of caspase 9 cleavage, an autocleavage activity which suitably controls the process of apoptosis (Twiddy and Cain 2007); an increase in Bax protein; and a decrease in Bcl-2 levels, that is a protein with anti-apoptotic effects and antagonist of tumor-suppressor genes (Qian et al. 2022). Regarding the PI3K/AKT/mTOR and STAT3 pathway, Arnicolide D successfully inhibited this route's fundamental proteins related to cell growth, cell proliferation, and metastasis by gene transcription, a critical factor in cancer progression (Paplomata and O'Regan 2014). Western blot showed a diminishing in every protein studied of this pathway, such as p-mTOR, mTOR, PI3K110 α, p-pI3Kp85, PI3Kp85, p-AKT, p-STAT3, and STAT3. Phosphorylated statuses were studied because the signaling is made through these posttranscriptional modifications (Afify et al. 2021). Qu et al. (Qu et al. 2020) experimented in vitro with the effects of Arnicolide D in two TNBC cell lines, one positive estrogen receptor and one positive progesterone receptor cell. They demonstrated that, as for above, the sesquiterpene lactone exhibited anti-proliferative effects in a dose- and time-dependent manner, showing inhibitory results in 24, 48, and 72 h, with doses going between 0.2 and 50 µM treatments; it is worth noting that these effects were better in TNBC cells. Anti-proliferative effects were further assessed by analyzing the cycle cell distribution. It was determined that the culture was arrested at the G2/M stage in a time-dependent manner. However, with differences between cell lines in doses, MDA-MB-468 culture was significantly arrested at a concentration of 2.5 µM, whereas MDA-MB-231 cells experienced cycle arrest with a treatment of 5 µM. Higher doses did not increase the cycle arrest rate (Qu et al. 2020). For its anti-apoptotic effect, it was shown that cells treated with 10 and 20 µM of Arnicolide D concentration for 24 and 48 h, significantly increased the rate of apoptotic cells, observed with chromatin condensation and formation of apoptotic bodies. Migration assays were also executed with MDA-MB-231 cells due to this cancer's augmented metastasis rate (Qu et al. 2020; Yin et al. 2020); it was concluded that cell migration was significantly suppressed at doses from 2.5 to 10 µM for 24 and 48 h. Finally, it was also reported that Arnicolide D inhibited key proteins in the Akt/mTOR signaling pathway and the STAT3 route. Qu et al. also implemented an in vivo model using xenografts of MDA-MB-231 in a BALB/c nude mice model, describing a decrease in tumor size and density with the oral treatment of 25 mg/kg and 50 mg/kg dose for 22 consecutive days; it did not caused any side effects, as seen as in the increase of body weight, cancer cells did not express the marker Ki67, associated with cell growth and proliferation (LI et al. 2015). Finally, the anticancer effects of Arnicolide D were evaluated by Huang et al. (Huang et al. 2014) in a human colon cancer cell line (HT-29). It was shown its anti-proliferative capability in a dose-dependent manner, achieving an IC_{50} of 2.9 µM in 72 h. Additionally, the anti-apoptotic effects were assessed, and it was reported that HT-29 cells were arrested at the G1 stage in a concentration of 3 µM for 72 h, as well as an increase in apoptotic cell counts. At the same concentration, but for 24 h, it was demonstrated that Arnicolide D increased and maintained constant levels of ROS for up to 4 h; this increase in oxidative molecules is related to cell death by mitochondrial damage and autophagy (Villalpando-Rodriguez and Gibson 2021), also exhibiting a decrease in nuclear NF-κB concentrations, as analyzed above (Table 2).

Recent studies have shown that the herb *C. minima* exerts very good anti-cancer effects against different tumors like tumors of the breast, colon, and nasopharyngeal cancer (Yao et al. 2022b).

Arnicolide D extracted from *C. minima* exhibited cytotoxicity on the human nasopharyngeal carcinoma cell line by inhibiting induced apoptosis, regulation of the cell cycle, and proliferation (Liu et al. 2019a); anticancer activity on triple breast cancer in vitro was demonstrated using the extract of *C. minima* prepared by ultrasonication (Lee et al. 2020a); also, *Arnica montana* whole plant extract prepared using ethanol revealed anticancer activity on breast cancer cells in vitro (Basu et al. 2022). *C. minima* extract was subjected to cytotoxicity assay against nasopharyngeal carcinoma cell line CNE-1 by MTT assay. Various concentrations of *C. minima* extracts (15–50 µg/ml) were used for the experiments, and cell lines were treated for different time intervals ranging from 24 to 72 h. The authors also studied the morphological changes in the cells, and cell cycle status was also evaluated. Western blotting was used to measure apoptosis and PI3K-Akt-mTOR signaling-related proteins. Authors claim that the extract effectively inhibited the proliferation of cell lines in a dose and time-dependent way, with significant morphological changes on the CNE-1 showing apoptosis. Significant observations were reported on the standard LO2 cell lines with a low cytotoxic effect.

Table 2 Preclinical studies that tested Arnicolide D as a potential anticancer compound

Cancer type	Experimental model	IC_{50}/dose	Molecular targets/ mechanisms	Outcomes	Reference
Melanoma	A375	The minimum dose that caused significant differences in cell viability, apoptotic rate, cell cycle and protein levels: 4 μM, 48 h	Inhibition of the IKK/IκBα/NF-κB p65 inhibition of phosphorylation of IKKα/β	Reduced cell viability and induction of apoptosis Elevation of protein levels of p53 and p21, as well as lowered protein levels of Cdc2 and Cyclin B1	(Zhu et al. 2019a)
	B16F10	Minimum dose that caused significant differences in cell viability and protein levels: 4 μM, 48 h		Reduced cell viability Elevation of protein levels of p53 and p21, as well as lowered protein levels of Cdc2 and Cyclin B1	
	C57/BL6 mice bearing B16F10 allografts	Dose: 4 mg/kg i.p., 15 consecutive days		Suppression of tumour growth. Lowered tumour volume and weight, no body weight loss	
Nasopharyngeal Carcinoma	CNE-1	IC_{50} (μM): 24 h: 3.31 48 h: 2.19 72 h: 1.76	Inhibition of key proteins of the PI3K/AKT/mTOR and STAT3 pathway	Cytotoxicity. Inhibition of cell proliferation in a time and dose-dependent manner Cellular morphology is like apoptotic cells Arresting in the G2/M checkpoint, and lower count of cells in G_1 phase Induction of apoptosis	(Liu et al. 2019a)
	CNE-2	IC_{50} (μM): 24 h: 4.26 48 h: 0.99 72 h: 0.83			
	SUNE-1	IC_{50} (μM): 24 h: 6.22 48 h: 0.66 72 h: 0.26			
	HONE1	IC_{50} (μM): 24 h: 9.97 48 h: 0.19 72 h: 0.02			
	C666-1	IC_{50} (μM) 24 h: 2.47 48 h: 0.91 72 h: 0.96			

Table 2 (continued)

Cancer type	Experimental model	IC_{50}/dose	Molecular targets/ mechanisms	Outcomes	Reference
Breast Cancer	MDA-MB-231	IC_{50} (μM): 24 h: 12.04 48 h: 5.211 72 h: 3.258	Inhibition of the activation of Akt/ mTOR and STAT3 pathway	Anticancer activity in a dose and time-dependent manner Cell cycle arrest in G2/M checkpoint Induction of apoptosis Inhibition of cell migration	(Qu et al. 2020)
	MDA-MB-468	IC_{50} (μM): 24 h: 9.507 48 h: 3.405 72 h: 2.515			
	MCF7	IC_{50} (μM): 24 h: 15.19 48 h: 9.083 72 h: 8.909		Cytotoxicity activity in a dose and time dependent manner	
	BALB/c nude mice model with MDA-MB-231 xenograft	25 or 50 mg/kg, p.o., 22 days	-	Reduction in tumour weight, volume and density, no body weight loss	
Colon Cancer	HT-29	IC_{50} (μM): 72 h: 2.9	Inhibition of NF-κB protein levels	Cytotoxicity Increase in apoptotic cells, staying at sub- and G1 phase Increased ROS formation	(Huang et al. 2014)

Akt, protein kinase B; *Cdc2*, cell division cycle protein 2; *CNE-1, CNE-2, SUNE-1, HONE1, C666-1*, nasopharyngeal carcinoma cell lines; Cyclin B1; *G1, G2/M*, cell cycle phases; *IC_{50}*, half maximal inhibitory concentration; *i.p.*, intraperitoneal; *IKKα/β*, IκB kinase alpha/beta; *IκBα*, inhibitor of kappa B alpha; *MDA-MB-231, MDA-MB-468, MCF7*, breast cancer cell lines; *mTOR*, mammalian target of rapamycin; *NF-κB*, nuclear factor kappa-light-chain-enhancer of activated B cells; *p.o.*, per os, orally; *p21*, cyclin-dependent kinase inhibitor 1; *p53*, tumor protein p53; *PI3K*, phosphoinositide 3-kinase); *ROS*, reactive oxygen species; *STAT3*, signal transducer and activator of transcription 3; *HT-29*, colon cancer cell line

On CNE 1 cells, cell cycle arrest was reported in the G2/M phase. The extract also exhibited inhibitory action on Bcl-2 expression, enhanced Bax, and activated caspase-3, 8, 9, and PARP in CNE-1 cells (Guo et al. 2015). In continuation of the study, the research group isolated different chemicals from the plant, Arnicolide D being one among them. The isolated compounds were tested on different nasopharyngeal carcinoma cell lines: CNE-1, CNE-2, SUNE-1, HONE 1, and C666-1. The authors concluded that Arnicolide D had the best inhibitory action against the cancer cell lines and was selected for further study. A network pharmacology approach, followed by a molecular docking study, was used to identify the most abundant active component of the herb against cancers, which can be targeted for treatment with *C. minima*. In vitro studies were carried out by the researchers to confirm the results obtained by the above findings further. The results from the study suggested that Arnicolide D was the most abundant component of the plant, which was responsible for the anti-tumor activity. Further, it was identified that prostate cancer contains approximately 42% of the targets, making it the most sensitive tumor for Arnicolide D. Further studies showed that Arnicolide D binds with Src with a binding energy of -7.3 kal/mol, making it the prime target, responsible for *C. minima* mediated anti-cancer activity. SIP (solvent-induced protein precipitation) assay, PCR, and the research group carried out Western blotting assays to validate these results. With these experiments, the authors recognized that *C. minima* or Arnicolide D did not affect the Src gene expression but suppressed the Src protein expression (Yao et al. 2022b). Active principles from *C. minima*, including Arnicolide D, exerted better cytotoxic activity against human colorectal adenocarcinoma cell line HT-29 than cisplatin. The study also reported that the cytotoxic action of Arnicolide D on normal cells was much weaker, which may result in fewer ADRs in clinical use. This also increased intracellular levels of reactive oxygen species and decreased levels of NF-ƙB, leading to cell cycle arrest in the G1 phase and apoptosis (Huang et al. 2014). NF-ƙB is identified as a molecular target in the treatment of melanoma, which is a lethal cancer. A study was carried out to assess the anti-melanoma efficacy of Arnicolide D, which involved in vitro and in vivo experiments. This study was undertaken since the compound Arnicolide D was known to bind the molecular target NF-ƙB in colorectal cancer. The study group used melanoma cell lines A375 and B16F10, using dacarbazine as a control in the MTT assay. In vivo, assay decreased the tumor weight by 53.7% compared to the control. In vitro studies showed a reduction in cell viability, G2/M cell cycle arrest, elevated levels of P53, and p21 decreased levels of Cdc2 and cyclin B1 in the cancer cells. The authors concluded that the mechanism of tumor suppression was through the inhibition of IKKα/β, IƙBα degradation, and phosphorylation of NF-ƙBp65 in melanoma cells (Zhu et al. 2019a).

Limitations and challenges

The development of Arnicolide D as a potential oncological agent faces several critical challenges. One significant limitation is the lack of comprehensive clinical data, as existing studies are primarily preclinical. This gap underscores the need for extensive clinical trials to ascertain its safety and efficacy in humans. Another challenge lies in fully elucidating the molecular mechanisms of Arnicolide D's action, which is important for its therapeutic application and understanding of potential side effects. The pharmacokinetic properties, including bioavailability and metabolism, also must be detailed investigation.

Conclusion and future perspectives

Arnicolide D, derived from *Centipeda minima* and other sources such as *Arnica montana* and *Arnica acaulis*, demonstrates extensive potential in traditional and modern medicine. In this comprehensive analysis, Arnicolide D, primarily extracted from *C. minima*, demonstrates a wide range of therapeutic applications; it exhibits significant neuroprotective and anti-viral activities against strains of influenza. Traditionally, in Asian and Ayurvedic medicine, it treats various conditions, including headaches, respiratory issues, and skin diseases. Recent scientific studies have expanded its scope, particularly highlighting its effectiveness in inhibiting cancer cell proliferation in breast, colon, and nasopharyngeal cancers, achieved through mechanisms such as cell cycle arrest and modulation of critical cellular signaling pathways.

Future research should focus on developing advanced drug delivery systems to optimize its therapeutic potential and minimize side effects. Additionally, exploring the synergistic effects of Arnicolide D with established cancer therapies could open new avenues in combinational treatments. Investigating its efficacy across a broader spectrum of cancer types would also be valuable, expanding its potential utility in oncology. While Arnicolide D holds considerable promise, addressing these challenges through rigorous scientific inquiry is essential for its successful transition from a laboratory finding to a clinical asset in cancer treatment.

Abbreviations A375: Human melanoma cell line; ADR: Adverse drug reaction; B16F10: Human melanoma cell line; Bax: BCL-associated X protein; Bcl-2: B-cell lymphoma 2; BRS3: Bombesin receptor subtype 3; *C. minima*: *Centipeda minima*; C666-1: Human nasopharengeal carcinoma cell line; cdc2: Cell division control-2; CE: Common era; CNE-1, CNE-2: Human nasopharyngeal carcinoma cell line;

CNS: Central nervous system; H1N1: Swine flu; H5N1: Avian flu; H9N2: A subtype of Influenza A; HONE 1: Human nasopharyngeal carcinoma cell line; i.p.: Intraperitoneal injection; LO2 cell: Human fetal hepatocyte cell line; mTOR: Mammalian target of rapamycin; NF-ƙB: Nuclear factor ƙB; PARP: Poly adenosine diphosphate-ribose polymerase; p-p65: Phosphorylated p65; p-IKKα/β: Phosphorylated IKKα/β; p-STAT3: Phosphorylated STAT3; p-mTOR: Phosphorylated mTOR; p-pI3Kp85: Phosphorylated pI4Kp85; p-AKT: Phosphorylated AKT; p-IκBα-p50-p65: Phosphorylated IκBα-p50-p65 complex; PI3K: Phosphatidylinositol-3-kinase; ROS: Reactive oxygen species; SUNE-1: Human nasopharyngeal carcinoma cell line; STAT3: Signal transducer and activator of transcription 3; TNBC: Triple negative breast cancer; u-p-IκBα: Ubiquitinated and phosphorylated IκBα

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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