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Article Review

Anticancer Activity of Asiatic Acid from *Centella Asiatica*: A Comprehensive Systematic Review of *In Vitro* and *In Vivo* Studies

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Abstract—*Centella asiatica, containing asiatic acid (AsA), represents one such candidate demonstrating promising anticancer effects. This study aims to comprehensively review the anticancer activity of AsA in published in vitro and in vivo studies. A systematic review method was employed and several databases, including Scopus, PubMed, ScienceDirect, and Google Scholar, were used to conduct a comprehensive and systematic search of the literature based in vitro and in vivo studies in November 2023 following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Thirty-three articles were included, distributed across twenty-five in vitro studies, three in vivo studies, and five both in vitro and in vivo studies. Based on the findings in the reviewed articles, we report that AsA, a triterpene derived from C. asiatica, exhibits anticancer effects demonstrated both in various cancer cell lines and in cancer cell-induced animal model, through several mechanisms, including anti-inflammatory effects, antioxidant effects, inhibition of cell proliferation, inhibition of invasion and migration, and induction of apoptosis and autophagy. Conclusions based on findings in in vitro and in vivo studies, AsA has strong potential to be used and developed as an inhibitor of various types of cancer cells.*

Keywords: *anticancer, asiatic acid, cancer, centella asiatica, herbal*

Abstrak—*Centella asiatica*, yang mengandung asam asiatik (AsA), mewakili salah satu kandidat yang menunjukkan efek antikanker yang menjanjikan. Penelitian ini bertujuan untuk meninjau secara komprehensif aktivitas antikanker AsA dalam studi *in vitro* dan *in vivo*. Metode yang digunakan adalah tinjauan sistematis pada beberapa basis data, termasuk Scopus, PubMed, ScienceDirect, dan Google Scholar, untuk melakukan pencarian literatur yang komprehensif dan sistematis berdasarkan studi *in vitro* dan *in vivo* yang dilakukan pada November 2023 dan mengikuti pedoman *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA). Tiga puluh tiga artikel diinklusikan, terdiri dari dua puluh lima studi *in vitro,* tiga studi *in vivo*, dan lima studi *in vitro* dan *in vivo*. Berdasarkan temuan dalam artikel yang ditinjau, kami melaporkan bahwa AsA, triterpen yang berasal dari *C. asiatica*, menunjukkan efek antikanker baik di berbagai sel kanker maupun pada model hewan yang diinduksi sel kanker, melalui beberapa mekanisme, termasuk efek antiinflamasi, efek antioksidan, penghambatan proliferasi sel, penghambatan invasi dan migrasi, serta induksi apoptosis dan autofagi. Kesimpulan berdasarkan temuan dalam studi *in vitro* dan *in vivo*, AsA memiliki potensi yang kuat untuk digunakan dan dikembangkan sebagai penghambat berbagai jenis sel kanker.

Kata kunci: *antikanker, asam asiatik, kanker, centella asiatica, herbal*

INTRODUCTION

Cancer is a disease related to abnormal and uncontrolled cell growth or proliferation, failure to differentiate, the ability to invade surrounding normal tissue, and has the capacity to spread as metastases to other organs [1]. Cancer remains a disease that demands more attention because its prevalence is still high. Approximately 19.3 million new cancer cases are occurred globally in 2020, with nearly 10 million mortalities caused by cancer, with the most

prevalent types of cancer being breast cancer (11.7%), followed by lung cancer (11.4%), colorectal cancer (10.0%), prostate cancer (7.3%), and gastric cancer (5.6%) [2].

Cancer is a disease influenced by multiple factors, in general, including tobacco and alcohol consumption [3], human papilloma virus [4], hepatitis viruses [5], radiation exposure [6], HIV/AIDS [7], marijuana use [8], Li Fraumeni syndrome [9], Fanconi anemia [10], xeroderma pigmentosum [11,12], diabetes [13], chronic inflammation [14], obesity [15], and unhealthy diet and lifestyle [16]. These diverse factors contribute to cancer through various biological pathways, many of which are interconnected. Chronic inflammation, for instance, creates a pro-tumorigenic environment by promoting DNA damage and sustaining abnormal cellular signaling [17]. Similarly, oxidative stress resulting from metabolic imbalances or external exposures induces genetic mutations and epigenetic changes that facilitate uncontrolled cancer cell proliferation [18]. Over time, these cells gain the ability to invade neighboring tissues and migrate to distant organs, a process known as metastasis. Furthermore, cancer cells often evade normal regulatory mechanisms such as apoptosis, enabling them to survive and grow under adverse conditions [19]. In some cases, they manipulate autophagy to meet their metabolic demands, further enhancing their survival and progression [20,21]. Understanding how these factors interact provides crucial insights into the complex mechanisms driving cancer development and progression.

Commonly employed cancer treatments encompass surgical procedures, chemotherapy, and radiotherapy [22]; however, they have adverse effects that worsen the quality of life of cancer patients [23]. Therefore, research continues to be carried out regarding alternative cancer treatments with as few side effects as possible, one of which is by using natural plant-based products. Plant-based medicines are reported to have bioactivity in inhibiting and curing several diseases, including cancer [24].

Centella asiatica (Linn.) Urban is a plant that can be found almost all over the world and is used as a dietary, beverage and medical resource because of the various properties it contains [25]. Various bioactivities of *C. asiatica* have been reported, including anti-inflammatory, anticancer, immunostimulant, anti-psoriatic, hepatoprotective, neuroprotective, anticonvulsant, antidiabetic, sedative, cardioprotective, antiviral, antifungal, antibacterial, antioxidant, insecticidal and wound healing activities [26–28]. These activities are obtained from 139 secondary metabolites found in *C. asiatica*, with one of the most commonly found compounds being asiatic acid (AsA) [27].

AsA, a natural pentacyclic triterpenoid, is often found in *C. asiatica* [29]. Anticancer, antidiabetic, anti-inflammatory, neuroprotective, cardioprotective, hepatoprotective, wound healing, bone and joint health, and respiratory health are the effects resulting from AsA [30]. One of the bioactivities of AsA is as an anticancer through reducing inflammation, cancer cell proliferation, and inducing the mitochondrial apoptosis pathway [31]. Preclinical studies have documented the anticancer effects of AsA through multiple mechanisms, including modulating important cancer-related signaling pathways, including phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), mammalian target of rapamycin (mTOR), p70S6K, and signal transducer and activator of transcription 3 (STAT3) [31,32]. In addition, further evidence suggests that AsA exhibits anticancer effects through molecular targets that play essential roles in tumorigenesis, proliferation, and metastasis of cancer cells, such as nuclear factor kappa B (NF-κB), tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF) [32].

In the present systematic review, the objective is to comprehensively discuss and evaluate the anticancer activity of AsA on several types of cancer cells focusing on published studies conducted in *in vitro* and *in vivo*, as well as to elucidate the mechanisms by which AsA hinders the development of cancer cells. This study provides a comprehensive review as well as identifies and clarifies the molecular mechanisms of AsA's anticancer activity. The findings in this study are expected to be an important step in understanding how AsA works and can be optimized in cancer therapy.

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METHODS

Review Methodology

We used a systematic review method in conducting this research, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The P (Population), I (Intervention), C (Comparison), O (Outcomes), and S (Study) framework was used in conducting this systematic review, with P: human cancer cell line and cancer-induced animal model, I: asiatic acid from *Centella asiatica*, C: no treatment control, placebo control, or standard cancer treatment, O: anticancer activity and mechanism of action, and S: *in vitro* and *in vivo* studies.

Information Sources and Search Strategy

A comprehensive search was carried out on the following databases: Scopus, PubMed, ScienceDirect, and Google Scholar in November 2023. In conducting a literature search on the specified databases, we used several search terms as follows: “asiatic acid” AND “Centella asiatica” AND (“cancer” OR “tumor” OR “anticancer” OR “antitumor” OR “cytotoxic” OR “cell line” OR “animal cancer” OR “human cancer”).

Eligibility Criteria

In selecting articles, we limited studies written in English, *in vitro* and *in vivo* studies, and articles that focused on discussing the anticancer effects of AsA from *C. asiatica*. All studies published from inception to November 2023 were considered for review in this research. No publication year restrictions were applied. We excluded all types of review articles, studies that discussed other than the anticancer activity of AsA, as well as articles that used AsA derivatives treated cancer cell.

Risk of Bias Assessment

We further performed a risk of bias assessment to assess the quality of the included studies. In this study, we used the Quality Assessment Tool for In Vitro Studies (QUIN) tool to assess the quality of *in vitro* studies with a score of 2 for adequately specified, 1 for inadequately specified, and not specified was scored as 0. Conclusions of study quality were assessed based on the total score, where scores >70%, 50-70%, and <50% were for low, moderate, and high risk of bias, respectively [33]. Meanwhile, for in vivo studies, the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE's RoB) tool was used, with "Yes", "Unclear", and "No" given for low, unclear, and high risk of bias, respectively [34]. We assigned a score of 1 for “Yes” and a score of 0 for “Unclear” and “No”, assessing low, moderate, and high risk of bias for the final results with scores of 8-10, 4-7, and 0-3, respectively. Studies that had mixed in vitro and in vivo methods were assessed using both quality assessment tools. Quality assessment of these studies was carried out by two reviewers (FMR and AJS), and validated by a third reviewer (AS). If there were differences of opinion in this process, discussions and decision making were carried out carefully.

Data Extraction

Finally, we extracted data from all included studies and presented them in a table containing important information, such as study methods, cancer cells/animal models, dosage, results in the form of anticancer activity, risk of bias of the study, and reference.

RESULTS

Selection Process

After identifying using several combinations of determined keywords in the databases, the results obtained were 3,515 records. Duplicates were removed with the aid of the Mendeley reference manager leaving 3,115 articles. As a result of reviewing titles and abstracts, 3,066 studies were excluded based on irrelevance and resulted in 49 reports for

further feasibility assessment. Finally, a total of 33 articles were included in this review. The entire selection process is depicted in *Figure 1*.

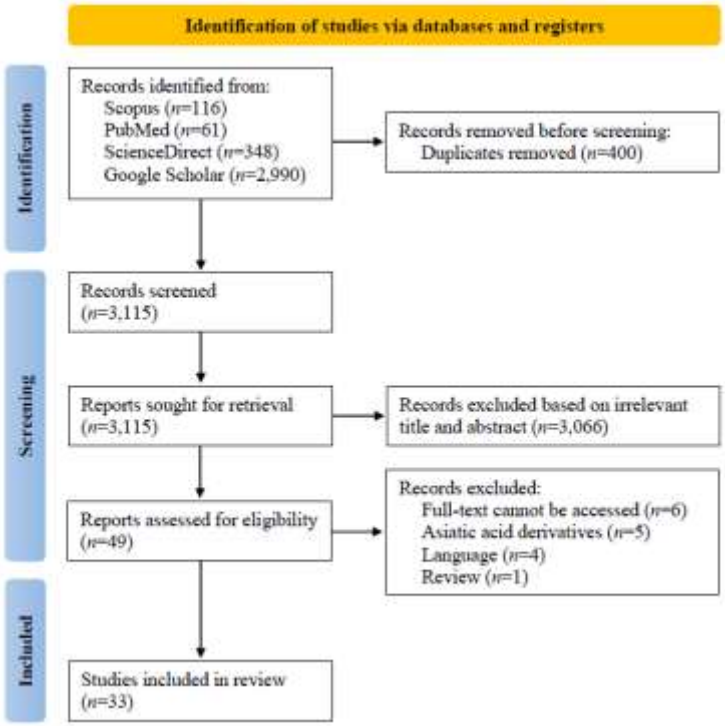

Figure 1. PRISMA flowchart.

Characteristics of Included Studies

A total of 33 articles were eligible, consisting of 25 *in vitro* studies, 3 *in vivo* studies, and 5 both *in vitro* and *in vivo* studies. The risk of bias assessment that has been conducted is also described in this section. Finally, all characteristics of the included studies are presented in Table 1.

Table 1
Summary of Anticancer Activity of AsA

Study Method	Cancer Cell(s) and/or Animal Model	Dose	Anticancer Activity	Risk of Bias	Reference
In vitro	Human hepatoblastoma HepG2 cell line	10, 20, 30, 40, 70, and 100 µM	Induces apoptosis through increasing intracellular Ca^{2+}. Increases p53 expression.	75.0% (Low)	[35]
	Human colonic adenocarcinoma HT-29 cell line	10-60 µg/mL	Increases tumor sensitivity to anticancer medications and decreases the negative effects of chemotherapy.	70.8% (Low)	[36]
	Human breast cancer MCF-7 and MDA-MD-231 cell lines	0, 2.5, 5, 10 and 20 µM	Suppresses cell proliferation and cell cycle progression. Triggers apoptosis.	66.7% (Moderate)	[37]
	Human melanoma SK-MEL-2 cell line	0, 10, 20, 30, 40, and 50 µM	Activates apoptosis through the formation of ROS, changes in the Bax/Bcl-2 ratio and caspase-3 activation.	66.7% (Moderate)	[38]

Study Method	Cancer Cell(s) and/or Animal Model	Dose	Anticancer Activity	Risk of Bias	Reference
	Human glioblastoma U87MG cell line	10, 20, 30, 40, 50, and 60 µmol/L	Induces apoptosis. Induces signaling involving Ca^{2+}.	66.7% (Moderate)	[39]
	Human colon cancer SW480 cell line, human stomach cancer SNU668 cell line, and murine colorectal adenocarcinoma CT26 cell line	0-50 µg/mL	Induces apoptosis through the mitochondrial death apoptosis cascade.	75.0% (Low)	[40]
	Human multiple myeloma RPMI 8226 cell line	10, 20, 30, 40, 50, 60, and 70 µmol/L	Decreases FAK expression levels.	75.0% (Low)	[41]
	Human hepatocellular carcinoma HepG2 and 293T cell lines	0, 5, and 10 µM	Enhances $p21^{WAF1/CIP1}$ stability by attenuating NDR1/2 dependent phosphorylation. Inhibits NDR1/2 kinase expression.	62.5% (Moderate)	[42]
	Non-small cell lung carcinoma A549 cell line	0, 25, 50, 75, and 100 µM	Upregulating miR-1290.	58.3% (Moderate)	[43]
	Human Chang liver HepG2 cell line	10, 20, 30, 40, and 50 µg/mL	Inhibits the growth of cancer cells.	37.5% (High)	[44]
	Human leukemia HL-60 cell line	10, 20, 30, 40, 50, and 60 µmol/L	Inhibits Bcl-2, survivin and MAPK signaling pathways.	75.0% (Low)	[45]
	Human hepatocellular carcinoma HepG2 cell line	25, 50, 75, and 100 µM	Causes ATP depletion, cytochrome C release, rapid cell death, and mitochondrial membrane potential dissipation.	66.7% (Moderate)	[46]
	Human ovarian cancer SKOV3 and OVCAR-3 cell lines	0, 10, 20, 40. 60, 80, and 100 µg/mL	Inactivates the PI3K/Akt/mTOR signaling.	75.0% (Low)	[47]
	Human liver hepatocellular carcinoma HepG2 cell line	3, 6, 12, 24, 48, and 96 µM	Counteracts t-BHP-induced cellular damage and oxidative stress via regulating NRF2 signaling through activating Akt and ERK expression.	75.0% (Low)	[48]
	Human metastatic ovarian cancer SKOV-3 cell lines	1, 3, 10 and 30 µM	Suppresses cell migration and invasion.	79.2% (Low)	[49]
	Human colon cancer SW480 and HCT116 cell lines	0-50 µg/mL	Inhibits proliferation and migration. Regulates Pdcd4 through the PI3K/Akt/mTOR/p70S6K signaling pathway.	66.7% (Moderate)	[50]
	Human cholangiocarcino	20-55 µM	Induces apoptosis through downregulation of anti-	75.0% (Low)	[51]

Study Method	Cancer Cell(s) and/or Animal Model	Dose	Anticancer Activity	Risk of Bias	Reference
	ma KKU-156 and KKU-213 cell lines		apoptotic genes. Increases early and late apoptotic cells.		
	Human lung cancer A549 cell line	5, 10, 20, 40, and 80 µmol/L	Increases E-cadherin. Inhibits Snail, N-cadherin and vimentin.		
	Human pancreatic cancer MIA PaCa-2 and PANC-1 cell lines	0, 20, 40, and 60 µM	Induces apoptosis and autophagy through AMPK activation and mTOR inhibition. Decreases expression of miR-17 and miR-21.	62.5% (Moderate)	[52]
	The cisplatin-resistant nasopharyngeal carcinoma NPC-BM and NPC-039 cell lines	0, 25, 50, and 75 µM	Induces apoptosis through p38 phosphorylation.	66.7% (Moderate)	[53]
	Human osteosarcoma HOS and 143B cell lines	0, 2.5, 5, 10, 20, 25, 30, 40, 50, and 100 µM	Induces apoptosis by suppressing the JAK2/STAT3 pathway and MCL-1 expression.	75.0% (Low)	[54]
	Doxorubicin-resistant breast cancer MCF-7 cell line	0-160 µM	Forms ROS. Reduces ATP content. Balancing adaptive immunity through intrinsic apoptosis, AMPK, PD-L1, and NF-κB.	75.0% (Low)	[55]
	Human lung adenocarcinoma A549 cell lines	Not available	Increases apoptotic activity. Downregulates MALAT1, p300, β-catenin and MDR1 expression. Upregulates miR-1297 expression. Decreases β-catenin nuclear translocation.	66.7% (Moderate)	[56]
	Human prostate cancer 22Rv1, PC3, and DU145 cell lines	0, 5, 10, 20, and 30 µM	Effects on Snail expression. Suppresses the MEK3/6-p38/MAPK signaling pathway through destroying MZF-1 and Elk-1 binding interactions.	79.2% (Low)	[57]
	Human nasopharyngeal carcinoma SUNE5-8F and TW01 cell lines	0-100 µM	Inhibits migration. Induces caspase-3. Inhibits STAT3 phosphorylation. Reduces claudin-1 expression.	70.8% (Low)	[58]
In vivo	TPA-mediated skin tumorigenesis in DMBA-initiated female ICR mice	30 µM and 50 µM (topical administration)	Inhibits NO and COX-2 signaling.	6 (Moderate)	[59]
	DMH-induced colon carcinogenesis male Albino Wistar rats	4 mg/kg (per oral)	Detoxifies carcinogens and reduces preneoplastic lesions based on anti-inflammatory, antiproliferative and pro-apoptotic effects.	8 (Low)	[60]
	Male nude mice	50 and 100 mg/kg,	Enhances the effect of antitumor of cisplatin by	5 (Moderate)	[61]

Study Method	Cancer Cell(s) and/or Animal Model	Dose	Anticancer Activity	Risk of Bias	Reference
		(injected intra-peritoneally)	inducing apoptosis and inhibiting cell proliferation.		
In vitro *In vivo*	Glioblastoma multiforme LN18, U87MG, and U118MG cell lines	10-100 µM	Modulates caspases, Bcl-2 family members, and survivin. Induces GRP78 and Calpain and decreases Calnexin and IRE1α expression. Increases Ca^{2+} and damages cellular organization.	62.5% (Moderate)	[62]
	Athymic (nu/nu) male nude mice	30 mg/kg/d (per oral twice a day)	Reducing tumor volume in mice.	5 (Moderate)	
	Human lung cancer A549 and H1299 cell lines	10, 20, 40, 60, and 80 µmol/L	Induces apoptosis. Decreases p62 expression. Collapses mitochondrial membrane potential and forms ROS.	75.0% (Low)	[63]
	Mouse Lewis lung cancer cells-injected C57BL/6J mice	50 and 100 mg/kg (per oral)	Inhibits tumor volume and weight. Significantly increases apoptotic activity.	6 (Moderate)	
	Human tongue cancer Tca8113 cell line	0, 10, 20, 30, 40, 50, 80, and 100 µM	Increases Ca^{2+} levels and Calpain expression. Promotes activation of the Grp78/IRE1α/JNK pathway.	79.2% (Low)	[64]
	Male BALB/cANNCjr nu/nu mice	15 mg/kg/d (injected intra-peritoneally)	Significantly reduces tumor volume and tumor weight.	5 (Moderate)	
	Human renal cell carcinoma 786-O, A-498, Caki-1, and ACHN cell lines	0-50 µM	Inhibits metastatic properties by inhibiting the p-ERK/p-p38MAPK pathway and downregulates MMP-15.	62.5% (Moderate)	[65]
	Male C. B17/SCID mice	25 mg/kg and 50 mg/kg (per oral twice a week)		5 (Moderate)	
	Human osteosarcoma 143B, MG63, HEB, HS5 and LO2 cell lines	0, 30, 35, 40, 45, 50, and 55 µM	Inhibits proliferation, migration and invasion. Induces apoptosis by inhibiting PI3K/Akt and NF-κB signaling.	66.7% (Low)	[66]
	BALB/c nude mice	20, 25, and 50 mg/kg (intra-gastrically, once every 2 days)	Inhibits cancer growth and the size of lung metastases.	7 (Moderate)	

DISCUSSION

Anti-inflammatory Effect

Tumor-infiltrating mast cells specifically change the nature and phenotype of Treg from anti-inflammatory to pro-inflammatory, resulting in chronic inflammation and cancer development [67]. AsA (4 mg/kg) exhibits anti-inflammatory by reducing the number of mast cells in rats [60]. In addition, 50 mg/kg and 100 mg/kg of AsA, it downregulates collagen (Col) I expression, Col IV, fibronectin, α-smooth muscle actin (α-SMA), and transforming growth factor (TGF)-β1. Furthermore, AsA also reduces mRNA and TGF-β1 expression in the kidney [61].

AsA decreases proteins related to inflammation due to long-term cisplatin treatment in HK-2 cells in nude mice, such as apoptosis-associated speck-like protein containing a CARD (ASC), NLR family pyrin domain containing 3 (NLRP3), and caspase-1. In addition, AsA also inhibits kidney injury molecule-1 (KIM-1), receptor interacting protein kinase 1 (RIPK1), and phosphorylated mixed lineage kinase domain-like (p-MLKL) expression in HK-2 cells treated with cisplatin [61].

In vivo investigations demonstrated a reduction in the expression of cyclooxygenase-2 (COX-2), indicating the presence of anti-inflammatory activities in ICR mice [59]. Because COX-2 is a pro-inflammatory enzyme, it is frequently expressed in various types of cancer [68,69]. Therefore, it can be concluded that AsA is effective in inhibiting cancer cells through inhibiting inflammation in several *in vivo* studies conducted.

Antioxidant Effect

AsA has an antioxidant effect on cancer cells. In HepG2 cells, AsA significantly activates total nuclear factor erythroid 2-related factor 2 (NRF2) protein expression and Kelch-like ECH-associated protein 1 (KEAP1) degradation [48]. KEAP1 binds NFR2, where NFR2 functions to regulate a particular set of target genes that encode enzymes or proteins that are important for antioxidant and detoxification responses, and significantly suppresses cancer cell growth [70,71]. Additionally, NRF2 activation causes increased antioxidant proteins and enzymes expression, including hemeoxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase-1 (NQO-1), and glutamate-cysteine ligase catalytic subunit (GCLC) [72,73], in accordance with the findings in the study conducted [48]. Finally, AsA possesses the capacity to inhibit cancer cells through activation of the NRF2 protein and degradation of KEAP1, which results in increased antioxidant proteins and enzymes expression.

Cell Proliferation

AsA inhibits cellular proliferation and progression in MCF-7 and MDA-MB-231 cells, resulting in a higher proportion of cells in the S-G2/M phase [37]. At a dose of 20-40 µg/L, AsA shows antiproliferative effects in SW480 cell line [40]. In RPMI 8226 cells, AsA considerably enhances the suppression of cell proliferation, resulting in a notable increase in the proportion of cells in the G2/M phase [41]. Observed in SKOV3 and OVCAR-3 cells, the suppression of cell growth is also achieved by an elevation in the proportion of cells in the G0/G1 phase, together with a decrease in the percentage of cells in the S and G2/M phases [47]. Additionally, G2/M phase blocking also occurs in 143B and MG63 cell lines [66].

Focal adhesion kinase (FAK) holds great potential as a cancer treatment target due to its association with cancer progression, invasion and drug resistance [74]. AsA (35 and 40 µmol/L) significantly impacts the inhibition of FAK and p-FAK expression in RPMI 8226 cell line [41], causing inhibition of proliferation and spread of cancer cells.

Proliferating cell nuclear antigen (PCNA) regulates the progression of the cell cycle and is involved in DNA replication, making it crucial for the rapid growth of tumor cells [75,76]. The analysis results showed that AsA inhibits PCNA in U87MG xenografts [62], in DMH-treated rats [60], and in 143B and MG63 cells, as well as in osteosarcoma tumors cell [66]. Meanwhile, Ki-67 is a marker of tumor cell proliferation [77], which is inhibited by AsA in U87MG xenografts [62] and renal cancer cells in C. B17/SCID mice [65].

PI3K, Akt, and mTOR phosphorylation levels decrease in cells treated with AsA, indicating that AsA inactivates the PI3K/Akt/mTOR signaling [47]. Corroborated by a study carried out by Hao et al., AsA also decreases the levels of p-PI3K, p-Akt, p-mTOR, and p-p70S6K and increases the expression of programmed cell death 4 (Pdcd4), which has an impact on the antiproliferative effect by modulating Pdcd4 through the PI3K/Akt/mTOR/p70S6K signaling pathway in SW480 and HCT116 cell lines [50]. In addition, inhibition of PI3K/Akt and NF-κB signaling activity also occurs in osteosarcoma cells treated with AsA [66].

In testing through *in vivo* studies, tumor proliferation is effectively suppressed by administering AsA (50 and 100 mg/kg), with no substantial occurrence of side effects [63]. Nude mice implanted with Tca8113 cells also showed a reduction in tumor volume and weight after being treated with AsA [64]. Furthermore, AsA also inhibits the growth of osteosarcoma and significantly reduces tumor density and shows nuclear pyknosis and nuclear fragmentation [66]. Based on these findings, it can be seen that AsA suppresses the growth of certain cancer cells by decreasing their proliferation.

Invasion and Migration

The inhibitory effect of AsA on cell invasion and migration was assessed in SKOV-3 cells by downregulating mesenchymal markers, such as vimentin, N-cadherin, and zinc finger E-box binding (ZEB)-1/2, and promoting epithelial markers such as E-cadherin, keratin (KRT)7, KRT14, and KRT19 [49]. The findings of study conducted in SW480 and HCT116 cells confirm that there is an increase in E-cadherin and a reduction in vimentin and N-cadherin, together with a reduction in the number of cells undergoing migration [50]. Similarly, the test results demonstrate a significant augmentation in E-cadherin and a reduction in N-cadherin, vimentin, and Snail in A549 cells as well as 143B and MG63 cells [66]. Apart from influencing the epithelial-mesenchymal transition (EMT)-related proteins expression, AsA is also efficacious in reducing the expression of extracellular matrix (ECM)-related proteins, including matrix metalloproteinase (MMP)-2 and MMP-9 in 143B and MG63 cell lines [66].

AsA reduces invasion and migration abilities in PC3, DU145 and 22Rv1 cells through suppressing the interaction of myeloid zinc finger-1 (MZF-1) and ETS like-1 (Elk-1) and inhibiting Snail mRNA expression. In addition, inhibition of invasion and migration also occurs by inactivating the mitogen-activated protein kinase kinase 3/6 (MEK3/6)-mitogen-activated protein kinase (MAPK)/p38 signaling pathway [57]. Significantly, AsA at a concentration of 40 μM inhibits the migration of TW01 and SUNE5-8F cells which resulted in approximately 50% inhibition of cell migration through decreasing N-cadherin and β-catenin, vimentin, and claudin-1, so AsA has the ability to regulate EMT markers [58].

Apoptosis

Administration of AsA induces apoptosis up to 3.9-fold and early necrosis up to 9-fold in U87MG cells [39], and increases early apoptosis in HL-60 cells significantly with levels from 4.58±2.01% to 25.68±5.67%. Meanwhile, the level of late apoptosis ranges from 11.20±2.91% to 60.63±6.65% [45]. The level of apoptotic cells also exhibited a 7- to 10-fold increase in SKOV3 and OVCAR-3 cells after administration of AsA (40 μg/mL) [47]. Hao et al. also reported that administration of AsA significantly increases the number of apoptotic cells and the intensity of apoptosis in SW480 and HCT116 cells [50], confirmed by research conducted by Zhu et al. which reported an increase in the number of apoptotic cells occurring from 8.7% to 32.95% [55]. In *in vivo* study conducted by Li et al. also reported that nude mice implanted with Tca8113 cells given AsA therapy shows more apoptotic cells [64].

Intrinsic and extrinsic pathways are the two main apoptotic pathways targeted by cancer therapy. A variety of proteins regulate both pathways, including pro-apoptotic proteins, BH3-specific pro-apoptotic proteins, and anti-apoptotic proteins [78]. AsA, among the pro-apoptotic proteins, possesses the ability to regulate the expression of these proteins. Increased Bax protein expression occurs in MCF-7 and MDA-MB-231 cell lines [37], 143B and

HOS cell lines [54], and SK-MEL-2 cell line [38]. A significant increase also occurs in Bad expression [62]. In SKOV3 and OVCAR-3 cell lines [47] and Tca8113 cell line [64], Bax expression increases after being given AsA. In cisplatin-resistant NPC-BM cells, AsA significantly increases Bax and Bak protein expression by 183% and 137%, respectively [53], and increases Bax and Bad expression in osteosarcoma cells [66].

Apart from regulating pro-apoptotic proteins, AsA also regulates anti-apoptotic proteins. This is proven by research which states that a significant decrease in Bcl-2 expression occurs in MCF-7 and MDA-MB-231 cell lines [37], 143B and HOS cell lines [54], SKOV3 and OVCAR-3 cell lines [47], and Tca8113 cell line [64]. Suppression of Bid and Bcl-2 expression is also reported to occur significantly [62]. Administration of 50 µg/mL AsA reduces the expression of Bcl-2 and Bcl-xL in HT-29 cell line [36]. Increasing Bax expression and decreasing Bcl-2 expression causes the Bax/Bcl-2 ratio to increase, as occurs in KKU-156 and KKU-213 cell lines [51]. Furthermore, inhibition of Bcl-2 expression occurs in MIA PaCa-2 cells after being given AsA [52], confirmed by *in vivo* research conducted by Siddique et al., that reduction in Bcl-2 expression also occurs in Wistar rats with DMH-induced colon carcinogenesis [60]. Thus, AsA has the ability to induce apoptosis through regulation of pro-apoptotic and anti-apoptotic proteins.

Caspases are categorized into two groups, namely initiator caspases which encompass caspase-2, -8, -9, and -10, and executor caspases, which consist of caspase-3, -6, and -7 [79,80]. Administration of AsA (50 and 100 µg/mL) sequentially activates caspase-3 in HT-29 cells up to 6.3-fold and 7.1-fold [36]. This study results are also supported by results reporting that AsA induces cleaved caspase-3 expression at a dose of 40 µM for TW01 cell line and 20 µM for SUNE5-8F cell line [58]. Increased caspase-3 activity also occurs in SK-MEL-2 cell line [38], U87MG cell line [39], SW480 cell line [40], MIA PaCa-2 cell line [52], Tca8113 cell line [64], and osteosarcoma cells both *in vitro* and *in vivo* [66]. Furthermore, AsA also activates caspase-8, -9, and -3 in LN18, U87MG and U118MG cell lines [62] as well as in cis NPC-039 and cis NPC-BM cells that are resistant to cisplatin [53]. Caspase-9 and -3 also increases in active amounts in SKOV3 and OVCAR-3 cell lines [47], A549 cell line [63], 143B and HOS cell lines [54], as well as in *in vivo* study conducted in DMH-induced colon carcinogenesis Wistar rats [60]. Caspase-3 and -7 are also reported to be activated in doxorubicin-resistant cells, MCF-7/DOX^R, after administration of AsA (80 µM) [55]. These data indicate that AsA stimulates the activation of initiator caspases, specifically caspase-8 and -9, as well as executor caspases, especially caspase-3 and -7.

In LN18 and U87MG cells, AsA increases the level of glucose-regulated protein 78 (GRP78), an indicator of endoplasmic reticulum (ER) stress. Apart from that, AsA also reduces inositol-requiring enzyme 1α (IRE1α) expression [62]. The research conducted by Li et al. demonstrates that AsA enhances the levels of GRP78, p-IRE1α, and phosphorylated c-Jun N-terminal kinase (JNK) in Tca8113 cells, without affecting the total levels of IRE1α and JNK proteins, thus AsA has the potential to activate the GRP78/IRE1α/JNK pathway [64]. As a result, AsA is able to significantly induce ER stress-mediated apoptosis.

AsA increases intracellular calcium (Ca^{2+}) concentration in HepG2 cell line [35,46], U87MG cell line [39], LN18, U87MB, and U11MG cell lines [62], and Tca8113 cell line [64]. The significant involvement of excessive mitochondrial Ca^{2+} accumulation is observed in the apoptotic process, especially in intrinsic apoptosis [81]. Increased Ca2+ uptake from the ER induces the activation of the permeability transition pore (PTP) by disrupting the mitochondrial membrane potential ($\Delta\psi_m$), which then induces apoptosis in cancer [82]. Furthermore, $\Delta\psi_m$ regulation is also regulated by AsA. At a concentration of 50 µmol/L, AsA prompts a loss of $\Delta\psi_m$ from 12% to 39% in U87MG cell line [39]. In SW480 cells, the induction of $\Delta\psi_m$ loss increases from 2.7% to 5.7-42% [40]. LN18, U87MG, and U118MG cells experienced a similar occurrence [62]. Wu et al. reported that AsA administration (20-80 µmol/L) for 12 hours causes a collapse of $\Delta\psi_m$ [63]. Therefore, it can be inferred that an elevated intracellular Ca^{2+} concentration leads to induction of PTP opening and disruption of $\Delta\psi_m$, regulated by AsA.

The stability of $\Delta\psi_m$ relies on adenosine triphosphate (ATP), where the higher the intracellular ATP level, the more stable the $\Delta\psi_m$ value [83]. Thus, to induce apoptosis, ATP levels must be reduced. In this case, AsA has the capability to regulate ATP, shown by the results of research conducted by Lu et al. which stated that at a concentration of 100 µM, AsA reduces ATP levels by up to 40% in isolated rat liver mitochondria [46]. Furthermore, during apoptosis, the loss of $\Delta\psi_m$ and cytochrome C (CytC) release from mitochondria results in the activation of caspase, fragmentation of DNA, and eventually, cell death [84].

CytC release is increased after administration of AsA to MCF-7 and MDA-MB-231 cell lines [37]. CytC is also released in SW480 cells following AsA treatment [40], HepG2 cell line [46], A549 cell line [63], and 143B and HOS cell lines [54]. In *in vivo* study, CytC release also occurs in DMH-induced colon carcinogenesis Wistar rats [60]. CytC release is essential for apoptosis process, resulting in apoptosomes generation, caspase activation, and cell death [85–87]. Thus, AsA effectively triggers apoptosis by promoting the release of CytC.

In addition, increasing intracellular Ca^{2+} concentration has an impact on a series of biochemical processes, including Calpain activation, where Calpain is also influenced by AsA. Increased Calpain activity occurred in U87MG cell line [39], LN18, U87MG, and U118MG cell lines [62], and Tca8113 cell line [64] after administration of AsA. Calpain activated by AsA then causes cellular damage and ends in the process of cancer cell apoptosis [88]. Additionally, Calpain is also associated with the apoptotic process by cleaving Bcl-2 family members, caspases, and apoptosis-inducing factors [89].

AsA increases the reactive oxygen species (ROS) formation. Corroborated by the results of Wu et al. which stated that AsA increases intracellular ROS in A549 cells, triggering mitochondrial dysfunction and ROS-dependent apoptosis [63]. Increased ROS causes damage to the mitochondrial membrane and releases CytC, then combines with Apaf-1 and pro-caspase-9 to create a complex, further causing DNA fragmentation [90,91]. DNA fragment formation forms in some cancer cells given AsA treatment. This statement is supported by research conducted by Hsu et al. which stated that DNA fragments were formed in MCF-7 and MDA-MB-231 cell lines [37]. Meanwhile, cells exposed to AsA exhibit characteristic apoptotic nuclear morphology, namely nuclear shrinkage, condensation and DNA fragmentation [40]. In addition, in cisplatin-resistant cancer cells, administration of AsA to cis NPC-039 and cis NPC-BM cells shows morphological characteristics of DNA fragmentation typical in apoptotic cells, and induces apoptosis in the early and late stages of apoptosis, especially at a concentration of 75 µM [53]. Hence, the formation of ROS is triggered by AsA, subsequently leading to the DNA fragmentation.

In MCF-7 and MDA-MB-231 cells, AsA increases $p21^{WAF1}$ expression [37]. Upregulation of $p21^{WAF1/CIP1}$ also occurs in HepG2 cells caused by inhibition of nuclear Dbf2-related kinase 1/2 (NDR1/2) expression [42]. Excessive expression of $p21^{WAF1/CIP1}$ results in caspase-3 activation and is associated with control of MAPK activation from the JNK family and p38 kinase [92]. In the MAPK signaling pathway controlled by $p21^{WAF1/CIP1}$, AsA itself also has the capability to modulate the MAPK signaling pathway. In cisplatin-resistant NPC-BM cells, administration of AsA (50 and 75 µM) significantly increases p-p38 activation by 298% and 438%, and decreases phosphorylated extracellular signal-regulated kinase 1/2 (p-ERK1/2) expression. Simultaneously, at a concentration of 75 µM, AsA increases p-JNK1/2 expression (Liu et al., 2020). Additionally, administration of AsA (10 µM) results in activation of p38 and ERK1/2 [37].

Survivin, an apoptosis inhibitor protein, is expressed in different cancer types and is linked to the degree of malignancy of a disease and poor prognosis [93]. AsA suppresses survivin expression in LN18 and U118MG cell lines [62], HL-60 cell line [45], as well as KKU-156 and KKU-213 cell lines [51]. Survivin inhibition results in increasing the rate of apoptosis, inducing Fas-mediated apoptosis, and increasing cell death caused by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Bax [94,95].

In addition, AsA significantly increases miR-1290 expression in A549 cell line [43](Kim et al., 2014). miR-1290 shows its activity in inhibiting cancer cells through regulating the Janus kinase (JAK)/STAT3, PI3K/Akt, Wnt/β-catenin and NF-κB signaling pathways which influence various pathways related to cancer cell inhibition, including apoptosis [96]. Furthermore, AsA significantly decreased the expression of MCL-1, p-STAT3, and p-JAK2, which allowed the induction of apoptosis in 143B and HOS cell lines via the JAK2/STAT3 signaling pathway [54]. According to findings from various literature, it is evident that AsA is a compound that possesses the potential to impede cancer cells through induction of apoptosis through various mechanisms.

Autophagy

Administration of AsA increases the generation of microtubule-associated protein 1 light chain 3 (LC3)-labeled vacuoles and increases the conversion of LC3-I to LC3-II in A549 cell line [63]. In MIA PaCa-2 cells, AsA increases the levels of LC3-II, p-p38, and phosphorylated AMP-activated protein kinase (p-AMPK), and reduces the levels of p-mTOR, consequently, the autophagy process ensues through the AMPK/mTOR signaling pathway [52]. More clearly, the autophagy regulation is involved in the expression of tumor suppressor proteins adversely controlled by mTOR and AMPK, ultimately leading to the initiation of autophagy [21]. Therefore, it can be deduced that AsA contributes to the inhibition of cancer cells through induction of autophagy as evidenced by *in vitro* studies.

CONCLUSION

In conclusion, we report based on the *in vitro* and *in vivo* findings in the reviewed articles that AsA, a triterpene derived from *C. asiatica*, has potential as an anticancer agent demonstrated both in various cancer cell types and in animal-induced cancer cells, through several mechanisms, including anti-inflammatory effects, antioxidant effects, inhibition of cancer cell proliferation, inhibition of invasion and migration, and induction of apoptosis and autophagy. The results of this systematic review provide an opportunity to conduct further research regarding the comparison of anticancer effectiveness and comparison of AsA with other compounds contained in *C. asiatica*. In addition, future research on AsA derivatives from *C. asiatica* may be warranted.

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