Literature Review: Cytotoxic Activity of Pineapple (*Ananas comosus* L.) Against Cancer Cells

Diyenda Ainun Nida and Haryoto(✉)

Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Jl. Achmad Yani Tromol Pos 1, Surakarta 57102, Pabelan Kartasura, Indonesia
har254@ums.ac.id

Abstract. Pineapple (*Ananas comosus* L.) is a plant in the *Bromeliaceae* family that has pharmacological effects such as antimicrobial, anthelmintic, diuretic, antidiabetic, and anticancer properties. This review aims to determine the anticancer activity and action mechanism of pineapple (*Ananas comosus* L.). The data sources for this review were articles published from 2012 to 2021, which were obtained from Google Scholar and PubMed databases with the keywords cytotoxic; *Ananas comosus* L.; cancer. The inclusion criteria used original articles or full-text research articles about the potential anticancer activity of pineapple (*Ananas comosus* L.) through in vivo and in vitro studies published between 2012 and 2021, while the exclusion criteria used were non-full text articles discussing the anticancer activity of pineapple (*Ananas comosus* L.). The search results on Google Scholar and PubMed search engines yielded a total of 594 articles, of which 468 articles were published within the last 10 years. Then, the articles obtained were identified, resulting in 8 articles that met the inclusion and exclusion criteria. The results of the analysis showed that pineapple (*Ananas comosus* L.) has anticancer activity from secondary metabolites, namely flavonoids and bromelain enzymes contained in pineapple (*Ananas comosus* L.). Bromelain and flavonoids in pineapple plants have anticancer activity by inducing apoptosis in cancer cells through p53 induction mechanisms, upregulation of Bcl-2 which is an anti-apoptotic protein, upregulation of Bax which is pro-apoptotic protein, induction of caspases, decreased expression of COX2, and regulating the MAPK and Akt/PKB pathway to inhibit NF-kB pathway.

Keywords: Ananas comosus L. · cancer · In vivo · In vitro

1 Introduction

Cancer is a disease characterized by uncontrolled cell growth caused by a malfunction in the cell regulatory mechanisms regulating proliferation and differentiation. The number of cancer patients continues to increase year after year and no effective treatment has been discovered, so cancer is a disease that is a serious concern in the medical field [1]. According to the Indonesian Ministry of Health [2], Indonesia (136.2/100,000 people)

© The Author(s) 2023
A. Sri Wahyuni et al. (Eds.): ICB-Pharma 2022, AHCPS 3, pp. 210–226, 2023.
https://doi.org/10.2991/978-94-6463-050-3_18

has the eighth highest cancer incidence in Southeast Asia and the 23rd highest in Asia. Lung cancer has the highest incidence rate per 100,000 people in Indonesia, at 19.4 people per 100,000, with an average mortality rate of 10.9 people per 100,000 in male patients, followed by liver cancer at 12.4 people per 100,000, with an average death rate of 7.6 people. Breast cancer has the highest incidence rate among women, with 42.1 population and an average mortality rate of 17 people [2], followed by cervical cancer with 23.4 population and an average death rate of 13.9 population. Cancer has long been one of the leading causes of death worldwide [3].

Radiotherapy and chemotherapy are the most common cancer treatments and are the most effective in curing cancer. However, this method has several drawbacks, including chemotherapy which can harm organs, such as the kidneys, lungs, and liver. Meanwhile, radiotherapy can cause cell and tissue damages as well as burns. Food ingredient, particularly fruit, is one of the supporting therapy attempts to conquer cancer. The biological activity of several foodstuffs, including fruits, has demonstrated its potential in inhibiting cancer cells, one of the fruits that can be used is pineapple (*Ananas comosus* L.) [3].

Ananas comosus L., commonly known as pineapple, is a monocotyledonous plant in the *Bromeliaceae* family. Pineapple has been extensively researched around the world to understand and explore its potential as an anticancer. Pineapple plants produce waste that is frequently disposed of because people only consume the fruit, which has a sweet taste. Waste from pineapple can be converted into products with added value. Wastes that can be utilized include leaves, skin, pineapple crown [4], and pineapple weevils that have not been optimally used [5]. A study conducted by [3] showed that pineapple fruit contains antioxidants from various phytochemicals such as phenolic compounds and flavonoids, where antioxidants work by capturing free radicals so that they can inhibit cancer cell proliferation and become anticancer agents. In addition, pineapple also contains chemical compounds such as the enzyme bromelain. Bromelain enzyme has antibiotic, antibacterial, anti-inflammatory, anticoagulant, antitumor, and anticancer properties [5]. Therefore, pineapple (*Ananas comosus* L.) has been widely studied to explore its potential as an anticancer agent.

Apoptosis, or programmed cell death, is a critical part of the cancer development process. Flavonoids can cause apoptosis by reducing DNA topoisomerase I/II activity, lowering Bcl-XL and Bcl-2 gene expression, decreasing reactive oxygen species (ROS), modulating signaling pathways, endonuclease activation, and increasing Bax and Bak gene expression [6]. In addition to flavonoids, the bromelain enzyme found in pineapple can induce apoptosis by inhibiting tumor cell proliferation and differentiation, as demonstrated in a study by Naritasari, *et al.* [7], which found that the ethanolic extract of pineapple weevil has anticancer activity against the human tongue squamous carcinoma cells through apoptosis induction.

The purpose of this review is to determine the anticancer activity of the pineapple plant (*Ananas comosus* L.) as well as the mechanism of secondary metabolite action or other compounds that can be isolated from the pineapple plant against cancer cells that have been tested and studied in previous studies and published in the available literature.

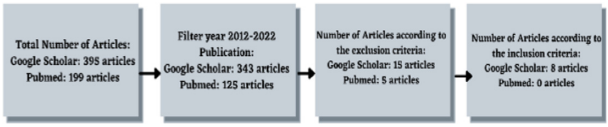

Fig. 1. Selection Stages

2 Method

The articles for the literature review were obtained from literature search databases such as Google Scholar and PubMed. The keywords used to search the articles were *Ananas comosus* L. or pineapple; cancer; anticancer activity; cytotoxicity. The articles obtained were then manually identified. Article eligibility was determined using inclusion criteria and exclusion criteria. The inclusion criteria were original articles or full-text research articles published between 2012 and 2021 that discussed the potential anticancer activity of pineapple (*Ananas comosus* L.) through in vitro and in vivo studies, while the exclusion criteria used were non-full text articles that discussed the anticancer activity of pineapple (*Ananas comosus* L.). The articles were then arranged, accompanied by information such as the author's name, year of publication, the media used in the experiment, and the final results of the research (Fig. 1).

3 Results and Discussion

The search results on the Google Scholar and PubMed search engines resulted in 594 articles, of which 468 articles were published within the last 10 years. The papers were then identified, and 8 articles matching the exclusion and inclusion criteria, as shown in Table 1 were acquired.

Gupta *et al.* [8] conducted a cytotoxic test using the MTT assay method on Ananas comosus L. fruit extract and a phytochemical analysis using gravimetric and colorimetric methods. The results revealed that *A. comosus* contains flavonoids, alkaloids, saponins, and tannins, with the highest flavonoid content, and has very low cytotoxic activity with IC_{50} values, namely IC_{50} cervical cells (HeLa): 53.41 ± 0.32 μg/mL; IC_{50} breast cancer cells (MCF-7): 50.82 ± 0.36 μg/mL; IC_{50} hepatocellular carcinoma cells (Hep G-2): 51.87 ± 0.29 μg/mL; IC_{50} bone sarcoma cells (MG-63): > 100.00 μg/mL. In 2015, Gani *et al.* [9] investigated the antiproliferative activity of pineapple flesh, core, and stem against ovarian and colon cancer cells. Pineapple is known to have antiproliferative activity obtained from bromelain. Bromelain has many health benefits, including immunomodulatory, digestive, anti-inflammatory, and anti-cancer properties. Isolation of bromelain was carried out using the precipitation method, the clarified pineapple juice was precipitated by adding 55% ammonium sulfate at 4ºC with constant stirring overnight. Bromelain extracted from the pulp, core, and stem of pineapple fruit was then tested for its anticancer properties using the MTT assay method. The results showed that pineapple had antiproliferative activity in cancer cell lines A2780 and HT29 at concentrations of 100 and 1000 μg/mL, with IC_{50} values in cancer cell lines A2780 of 280.54 μg/mL; 231.71 μg/mL; 280.54 μg/mL in the pulp, core, and stems of pineapple

Table 1. The results of a review of articles on anticancer activity in pineapple plants (*Ananas comusus* L.)

Reference	Plant parts	Cancer cells	Research result (μg/mL)
[8]	whole pineapple	HeLa, MCF-7, HepG2, MG-63	IC_{50} HeLa: 53,41 ± 0,32 IC_{50} MCF-7: 50,82 ± 0,36 IC_{50} HEP-G2: 51.87 ± 0.29 IC_{50} MG-63: > 100.00
[9]	Flesh, core, pineapple stem	A2780 dan HT29	IC_{50} A2780: flesh: 280, 54; core: 231, 71; stem: 280.54 IC_{50} HT29: stem: 267, 30; core: 324, 34
[10]	Pineapple pulp, skin, core and crown	MCF-7, A549, HCT116	Unfermented pineapple extract: IC_{50} MCF-7: 32,00 ± 3,50 IC_{50} A549: 30,72 ± 3,80 IC_{50} HCT116: 23,16 ± 3,22 Fermented pineapple extract: IC_{50} MCF-7: 27,43 ± 2,81 IC_{50} A549: 26,36 ± 2,90 IC_{50} HCT116: 19,33 ± 2,11
[11]	Pineapple leaves	SKOV-3 dan MCF	Leaf extracts grown in the field and in vitro showed low cytotoxic activity (<50% inhibition) against SKOV-3 and MCF-7 cancer cells.
[12]	Pineapple types of Pattavia and Nanglae	HepG2	IC_{50} nanas pattavia: 22,40 IC_{50}: nanas nanglae: 24, 28
.[3]	Whole pineapple flesh with crown	T47D	IC_{50}: 488, 00
[13]	Whole pineapple	T47D	IC_{50}: 741, 46
[14]	Whole pineapple	MCF-7, SKOV, HeLa	IC_{50}: MCF-7: > 100 IC_{50} SKOV-3: > 100 IC_{50} Hela > 100

for 24 h. Then the bromelain HT29 cancer cell line has an IC_{50} value of 267.30 μg/mL; 324.34 μg/mL on pineapple stems and core tested for 24 h. Based on the IC_{50} value obtained in the research conducted by Gupta *et al.* [8] and Gani *et al.* [9], according to Cho *et al.* (1998) in [15], the strength of anticancer activity in pineapple is very low because the IC_{50} value is > 30 μg/mL.

Then, in 2015, Rashad *et al.* [10] looked into the anticancer activity of pineapple waste, which is a by-product of the pineapple production industry composed of fermented and unfermented pulp, skin, core, and crown residues against the line of cancer cells

HCT116, MCF-7, and A549. The results showed that unfermented pineapple extract has anticancer activity against A549, MCF-7, and HCT116 with IC_{50} values, respectively, 30.72 ± 3.80; 32.00 ± 3.50; and 23.16 ± 3.22 μg/mL and the fermented pineapple extract has anticancer activity against A549, MCF-7, and HCT116 with IC_{50} values 26.36 ± 2.90; 27.43 ± 2.81; and 19.33 ± 2.11 μg/mL. Based on the IC_{50} value obtained in the research conducted by Rashad *et al.* [10], Cho *et al.* (1998) in [15] argued that the strength of anticancer activity in unfermented pineapple extract against MCF-7 and A549 cells showed very low activity because the IC_{50} value was > 30 μg/mL, while HCT116 cells showed moderate anticancer activity because the IC_{50} value was 11–30 μg/mL, meanwhile, the fermented pineapple extract showed moderate strength of anticancer activity in the three cell lines, because the IC_{50} value was 11–30 μg/mL.

Furthermore, Abdul Halim *et al.* [11] investigated the anticancer activities of pineapple leaves against human breast cancer (MCF-7) and ovarian cancer (SKOV-3) cell lines. Pineapple leaves were extracted using methanol as a solvent and then tested for phytochemical content and anticancer activity in pineapple leaves. The results showed that the phytochemical compounds found in pineapple leaves including flavonoids, tannins, alkaloids, and sterols, and pineapple leaf extract (*Ananas comosus* L.) showed low cytotoxic activity (<50% inhibition) against SKOV-3 and MCF-7 cancer cells. This is caused by low amounts of bioactive compounds in pineapple leaves, so pineapple leaves may not be suitable for use as chemotherapy agents. Then, in 2017, Khamenkhetkarn *et al.* [12] investigated the antiproliferative activities of pineapple Patavia and nanglae species from Northern Thailand against the HepG2 cancer cell line. The results of inhibitory concentrations at 50% (IC_{50}) of pattavia and nanglae extracts were 22.40 and 24.28 μg/mL. From these results, pattavia extract was more toxic to HepG2 cells than Nanglae. Based on the IC_{50} value, according to Cho *et al.* (1998) in [15], the pattavia and nanglae pineapple extracts showed moderate strength of anticancer activity with an IC_{50} value of 11–30 μg/mL.

Then, in 2020, Widyanto, Halimah, *et al.* [3] tested the cytotoxic activity of water extract of *Ananas comosus* L. against T47D cell model breast cancer cells by extracting it using water as a solvent and performing a cytotoxic test using the MTT assay method. The water extract of *Ananas comosus* L. is known to have low cytotoxicity with an IC_{50} value of 488.003 μg/mL. Nevertheless, Widyanto, Halimah, *et al.* [3] reported that pineapple has the potential as a chemopreventive agent in vitro against T47D breast cancer cells. This research was then continued by Widyanto and Putri *et al.* [13] in 2020 using methanol as a solvent in the extraction process, and the IC_{50} value which still had weak cytotoxicity was 741, 46 μg/mL. It is known that pineapple fruit water and methanol extracts can be used as chemopreventive agents against T47D breast cancer cells. It is because pineapple contains flavonoids, which can trigger apoptosis in cancer cells through downregulation of anti-apoptotic cells such as Bcl-XL and Bcl-2. Subsequent research was conducted by Tuy-On *et al.* [14] who examined the anticancer effect of pineapple extracted using 95% ethanol as solvent on MCF-7 (breast cancer), SKOV-3 (ovarian cancer), and HeLa (cervical cancer) cell line, obtaining IC_{50} results of MCF-7: > 100 μg/mL; SKOV-3: > 100 μg/mL; Hela > 100 μg/mL. Based on the IC_{50} value, Cho *et al.* (1998) in [15], stated that the strength of anticancer on these results indicates a very low anticancer activity because the IC_{50} value is > 30 μg/mL.

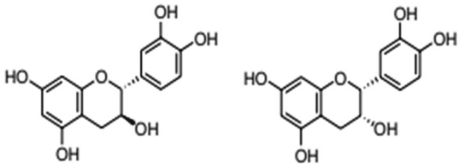

Fig. 2. Catechin (left), Epicatechin (right) [23].

3.1 Active Compounds in Pineapple Plants as Anticancer

The pineapple or *Ananas comosus* L. is a member of the *Bromeliaceae* family [16] that grows in Indonesia, Malaysia, Philippines, Thailand, Kenya, India, China, South America, and several other tropical and subtropical countries [10]. Several plants in this family are used in traditional medicine to treat various pathologies, with pharmacological action such as diuretic, antibacterial, anthelmintic, antidiabetic, antitussive, anticancer, antiproliferative, and pro-apoptotic [17, 18]. The secondary metabolites contained in pineapple (*Ananas comosus* L.) include flavonoids, saponins, tannins, alkaloids, phenols, and terpenoids [19, 20]. In addition to the phytochemical content above, pineapple also contains the enzyme bromelain, vitamin A, vitamin C, phosphorus, calcium, potassium, protein, sodium, iron, magnesium, and fiber, which are beneficial for body health [20].

3.1.1 Flavonoids

Flavonoids are polyphenolic compounds that are found in a variety of plants [21]. Flavonoids are most commonly found in fruits, spices, stems, cereals, nuts, vegetables, flowers, and seeds [22]. Flavonoids are made up of two benzene rings (A and B) linked by an oxygen-containing heterocyclic ring (C). Flavonoids are classified into different subclasses based on the structure of rings B and C. There are 6 types of flavonoids; flavanols, isoflavones, flavanones, flavones, flavonols, and anthocyanidins [21]. The flavonoid compounds found in pineapple include catechins and epicatechins (Fig. 2.) which are flavonoid compounds of the flavanol subclass [23]. The benefits of flavonoids have been widely used as anticancer, antitumor, anti-proliferative, antimicrobial, antiviral, antiangiogenic, antimalarial, and antioxidant [22]. Flavonoids have anticancer activity by inducing apoptosis and have anticancer activity through inhibition of cell proliferation by cessation of the cell cycle in the G2/M phase, apoptosis, and autophagy induction [24, 25]. The flavonoid content in pineapple extract is presented in Fig. 3. [26, 27].

3.1.2 Bromelain

Bromelain is an enzyme found in pineapple (Ananas comosus L.) fruit extract, consisting of a mixture of both non-protease and protease components [28]. Bromelain is known to inhibit tumor cells differentiation and proliferation, as well as to reduce the ability of cells to migrate and invade glioma cells and to reduce metastasis in lung cancer [7]. Bromelain also acts as an immunomodulator and cancer suppressor by increasing the formation of antibodies to fight cancer by increasing the cytotoxic activity of macrophages and monocytes. [18] (Table 2 and Fig. 4).

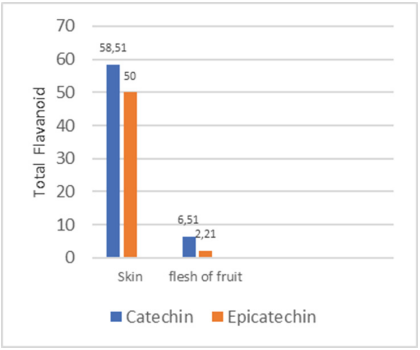

Fig. 3. The content of flavonoids in pineapple extract [26, 27].

3.2 Mechanism of Apoptosis Induction by Bromelain and Flavonoids in Pineapple Against Cancer Cells

3.2.1 Effects of Bromelain and Flavonoids in Inducing Apoptosis Through Extrinsic and Intrinsic Pathways

Apoptosis, or programmed cell death, is a critical step in the progression of cancer [6]. There are two types of apoptosis induction by the regulation of caspases. If the cellular signal that activates apoptosis is of extracellular origin, it is called the extrinsic pathway, but if the cellular signal is of intracellular origin, it is called the intrinsic pathway [37]. The extrinsic pathway is a death receptor-mediated pathway that involves the signaling complex, and the activation complex of procaspase 8 and 10, while the intrinsic pathway is a mitochondrial-mediated pathway through the apoptosome, and the activation complex of procaspase 9 [38, 39].

Extracellular signals are used by the extrinsic pathway to initiate apoptosis [35]. Extracellular ligands are a subset of extracellular signals [40]. The extrinsic pathway induces apoptosis by attaching external ligands such as tumor necrosis factor (TNF), TNF-related apoptosis inducing ligand (TRAIL), and Fas (Fas-L) to the extracellular domain of a transmembrane receptor or death receptor including TNF type 1 (TNFR1), Fas (CD95/Apo1), and TRAIL [41]. When an apoptosis signal binds to its death receptor or an extrinsic pathway through the corresponding extracellular domain of a transmembrane receptor on the plasma membrane, the death receptor is activated [38]. Each receptor can be activated independently by forming a death-inducing signaling complex (DISC) and recruiting intracellular death domains such as TNF receptor-associated death domain (TRADD), adapter Fas-associated death domain (FADD), and death effector domain (DED), which includes procaspases 8 and 10 [41, 42]. DISC induces autocatalytic activation of procaspase-8, whereas active caspase-8 effector caspases (caspase-3, -6, -7) can damage the nucleus and other intracellular structures, causing cell death [41]. Flavonoids in pineapple can cause apoptosis by increasing the expression of the proapoptotic gene, namely caspase-8. Its caspase-8 directly processes downstream effectors, namely caspase-3, -6, and -7, and in another pathway, caspase-8 can activate crosstalk pathways between the death receptor and the mitochondrial pathways through the cleavage of tBid to Bid [29].

Table 2. Anticancer Mechanism of Pineapple Plant

Metabolit-e name	Types of Cancer	In vivo/ In vitro	Mechanism of action	Reference
Flavonoids (catechins)	SiHa Cells	In vivo	Improved expression of the proapoptosis p53, upregulation caspase-3, caspase-9, and caspase-8 genes	[29]
	T47D and HFF cells	In Vitro	Inhibit nfkB, decrease COX-2 expression, increase ratio from Bax/Bcl-2, upregulation p53, caspase-3, caspase-9, and down -regulated PI3K, Akt	[30]
Bromelain	A431 cells and A375 melanoma cells	In vitro	Inhibition of NFkB activation, decrease in COX-2 expression, induction Bax/Bcl-2 ratio, induction caspase-9 and caspase-3, termination of G2/M phase cell cycle.	[31]
	mkn45 cells	In vitro	Caspase induction, split p53 induction supports the direct extranuclear apoptosis function of p53, inhibition of severed Akt, and decrease in Bcl-2	[32]
	Colorectal cancer (CRC)	In vitro	Block map kinase and (PI3K)/Akt signaling.	[33]
	MCF7 and MDA-MB-231 cells	In vitro	Upregulation of p53 and Bax, lowering the expression COX-2 and BcL-2, blocking NF-κB.	[34]

The extrinsic and intrinsic mechanisms converge after caspase-8 activation. Caspase-8 activation activates the death agonist of the BH3 interaction domain (BID), a BH3-only protein, in the extrinsic pathway. BID then activates, causing BAX and BAK to

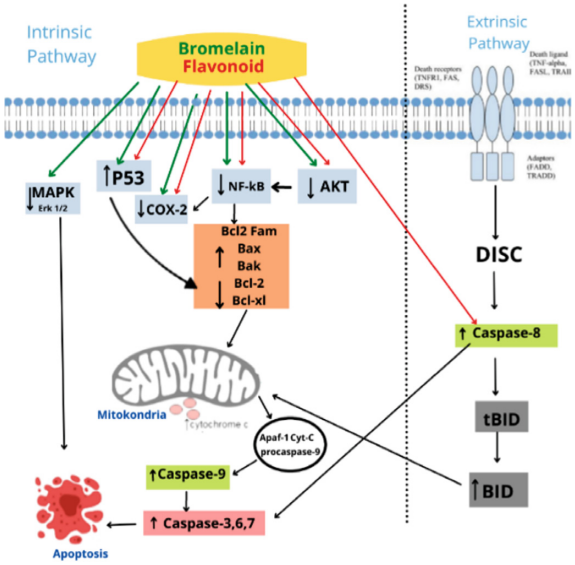

Fig. 4. *Apoptosis Induction Mechanism of Pineapple Plant* [30, 35, 36].

oligomerize and the intrinsic apoptotic process to continue. As a result, both routes continue to spread along their respective paths, confirming that apoptosis occurs [35].

The intrinsic route begins inside the cell. Internal stimuli such as irreversible genetic damage, hypoxia, high cytosolic Ca2 + concentrations, and oxidative stress, as well as the presence of damaged DNA cells or controlled oncogenes, are all triggers for the initiation of the intrinsic mitochondrial pathway. Aside from these triggers, the release of pro-apoptotic chemicals like cytochrome-c into the cytoplasm and increased mitochondrial permeability activate the intrinsic pathway. [35, 43]. The Bcl-2 protein family, which contains both antiapoptotic and proapoptotic activities, controls the whole system. [44]. There are two main groups in the Bcl-2 family, namely genes encoding anti-apoptotic proteins such as Bcl-2 and Bcl-XL and pro-apoptosis protein such as Bax and Bak [45] and by suppressing the pro-apoptotic Bcl-2 family members BAX and BAK, the anti-apoptotic Bcl-2 protein inhibits apoptosis. [44]. Anti-apoptotic proteins prevent mitochondrial cytochrome-c release. The balance of pro and anti-apoptotic proteins determines whether or not apoptosis occurs [45]. Members of the B2 cell lymphoma protein family (Bcl-2) are essential regulators of caspase-9 activation because they can inhibit or prohibit cytochromes from mitochondrion from entering the program and regulation of apoptosome formation. [37]. An apoptosome is formed when cytochrome c joins with Apaf-1 and procaspase-9. Apoptosomes are multi-protein complexes that contain a seven-spoke ring-shaped complex that activates caspase 9 and then the caspase-3 signaling cascade, resulting in cell death and apoptosis [46]. Bromelain and flavonoids in pineapple plants induce apoptosis Bax/Bcl-2 ratio by upregulating the pro-apoptotic and downregulating the anti-apoptotic protein such as Bax and Bcl-2, induction of caspase-9, caspase-3, and Apaf-1 expression in a variety of cancer cells [30-32, 34, 47].

Furthermore, intrinsic pathway includes extracellular signaling reception and activation of pathways such as phosphatidylinositol-3-OH kinase (PI3K)/AKT (protein kinase B), Ras-mitogen-activated protein kinase (MAPK)/extracellular signalling protein kinase (ERK) regulator, and COX-2 pathway to induce apoptosis[48].

3.2.2 Effects of Bromelain and Flavonoids in Inducing Regulation of P53

P53 is an important regulator of apoptosis and cell cycle arrest, loss of p53 increases tumorigenesis [37]. P53 involves in apoptosis because it can cause significant mitochondrial outer membrane permeabilization (MOMP), caspase enzymatic apoptosis, chromatin degradation, and cytochrome c release. The occurrence of mitochondrial outer membrane permeabilization (MOMP) is regulated by members of the pro-and anti-apoptotic Bcl-2 family [49]. Bromelain and flavonoids in pineapple plants can induce apoptosis through p53 upregulation mechanisms, by inducing the release of mitochondrial cytochrome c, p53 induction can promote the occurrence of direct extranuclear apoptosis of p53. Besides, p53 can activate effector caspases such as caspase-3 and other caspases [29, 30, 32, 34, 47].

3.2.3 Effects of Pineapple in Blocking MAPK Signaling and Akt/Protein Kinase B.

The mitogen-activated protein kinase (MAPK) is an important pathway for human cancer cell survival [50]. The MAPK pathway is a complex regulatory network of interconnected pathways that can affect a variety of physiological processes such as cell metabolism, growth, differentiation, and apoptosis [51, 52]. There are four independent MAPK pathways, the MAPK/ERK family or classical signaling pathways, and the Big MAP Kinase-1 (BMK-1), c Jun N-terminal kinase (JNK), and the p38 signaling families [50]. Bromelain can increase apoptosis-associated proteins while decreasing NF-B-driven Cox-2 expression in mouse skin cancers by blocking MAPK and Akt/protein kinase B signaling, [47].

3.2.4 Effects of Pineapple Plants in Lowering COX-2 Expression

COX-2 is linked to carcinogen generation, tumor development, angiogenesis inhibition, and metastatic processes. Overexpression of COX-2 can inhibit apoptosis and enhance the expression of the antiapoptotic protein Bcl-2 by activating the MAPK/ERK pathway [48, 53]. Inhibition of apoptosis will cause cancer cells to continue to grow so that increased COX-2 expression and increased anti-apoptotic protein Bcl-2 can block apoptogenic substances like cytochrome c out of mitochondria [53]. Bromelain induction of Cox-2 in mouse skin involves a number of transcription factors, including NF-kB [54]. Bromelain and flavonoids found in pineapple plants inhibit the activation of NF-kB, which reduces COX-2 expression. The bromelain-inhibited Akt cell survival protein may also be linked to NF-kB regulation [30, 31, 34, 47].

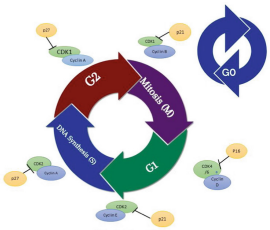

Fig. 5. Mechanism of Cell Cycle Inhibition by Bromelain [Adapted from [57].

3.2.5 Effects of Pineapple Plants in Inhibiting NF-KB

In mammals, the nuclear factor-k-light-chain-enhancer of activated B cells, also known as NF-kB, is a key modulator of the immunological and inflammatory responses. NF-kB was first discovered in activated B cells as a protein that interacts with the enhancer element of the immunoglobulin kappa light chain gene. The NF-kB pathway activates multiple target genes, including antiapoptotic proteins from the Bcl-2 family (Bcl-xL, Bcl 2), and has been demonstrated to protect cells from apoptosis [37]. Under certain conditions, NF-kB can also increase transcription of ras, c-myc, p53, and PKB/Akt which is a proapoptotic target gene [31]. Thus, the balance between pro-apoptotic and anti-apoptotic signaling components is key in the process of cell apoptosis [37]. NF-kB functions as a "signaling center" for increasing growth factors, cytokines, and inflammatory mediators, and it interacts with numerous other signaling pathways [31]. NF-kB can be activated through the expression of Akt, which can inhibit the apoptotic process [55]. Withdrawal of extracellular signaling molecules, oxidative and osmotic stress, irradiation, chemotherapeutic drug treatment, and ischemia shock have all been linked to Akt as an anti-apoptotic factor. Many cell types have anti-apoptotic effects when Akt is overexpressed, resulting in resistance or delayed cell death [56]. Bromelain and flavonoids can also induce apoptosis by downregulating NF-B by downregulating Akt [30, 31, 34, 47].

3.2.6 The Mechanism of Cell Cycle Inhibition by Pineapple Plants

The cell cycle is a complex stage found in eukaryotic cells that consists of the replication of the genetic model, timing of cell division, and protein-enzyme interactions [58]. This stage of the cell cycle consists of two different phases; the first is mitosis (M), which occurs when the cell undergoes cell division, and interphase, which includes the G1 phase, the S phase, and the G2 phase. After interphase, the cell returns to G0 (quiescence). In the G1 phase, the process of RNA and protein synthesis carried out to prepare DNA synthesis in the S phase is called the G1 phase or pre-DNA synthesis phase. Furthermore, the initial time until the cell's DNA replication is complete is called the S phase, after which genetic information will be transmitted to daughter cells from the M phase division through DNA replication to ensure stable genetic traits. Therefore, the S phase is the critical phase of the cell cycle. The G2 phase, also known as the pre-division phase, occurs between the completion of initial replication and the beginning of mitosis. During this time, RNA and proteins directly related to mitosis, such as tubulin, microfilaments, and

other important factors involved in mitosis regulation, are synthesized. The M phase takes place when the chromosomes are divided into two daughter cells. In the process of cell development and reproduction, the end of the previous cell cycle usually marks the beginning of the next cycle, although some cells do not enter the next cycle and instead briefly enter the G0 phase. Mitogens cause cells in the G0 phase to transition to the G1 phase [57, 59].

Several cyclin-dependent kinases (Cdks work in tandem with their cyclin partners to regulate the cell cycle. Mitogenic signals activate cyclin-dependent kinases (Cdks), which are important in cell cycle regulation and can be blocked by checkpoint activation in response to DNA damage [60]. Cyclin-dependent kinases (Cdks) have a serine/threonine-specific catalytic core that binds to the cyclin, a regulatory component that regulates substrate specificity and kinase activity [61]. The checkpoint is a sensor that monitors each phase of the cell cycle as well as the transition from one to the next. Checkpoints can prevent the transition from proceeding to the next phase until the previous one has been completed. The checkpoint's purpose is to identify any genetic fault repair, prohibit uncontrolled cell division, and ensure that both daughter cells inherit a complete copy of the genome. When a problem is detected, the checkpoint is activated, causing cell cycle arrest. When the problem is resolved, the checkpoint is deactivated, and if the repairs are not successful, the cell is driven towards senescence or apoptosis. Once the chromosomes have been correctly duplicated, the cell can enter G2, another cleft phase, to prepare for mitosis (M), [62].

The transition from the G2 phase to mitosis causes morphological and physiological changes in a proliferating cell. A network of the stimulator, inhibitory, and phosphatase protein kinases, led by Cdk1-cyclin B1, regulates the process of cells entering the mitotic phase [63]. Cylin B binds to Cdk1, controlling the M phase of the cell cycle [61]. Cyclin B1 is also critical for triggering the G2/M transition [63]. Cdk1 is one of the key kinases that drives cells into the mitotic phase, and its activation requires cyclin B binding and T-loop phosphorylation. The cyclin B-Cdk1 complex is inactivated by Myt1 and WEE1 phosphorylation of Thr14 and Tyr15 prior to the mitotic phase. Prior to mitosis, WEE1 and Myt1 are downregulated to allow Cdk1 activation. In mammalian cells, Myt1 inactivation is linked to Plk1 and Cdk1 phosphorylation [64].

To enter mitosis, cyclin B/cdk1 must be dephosphorylated by cdc25 phosphatase because cyclin B/cdk1 remains inactive until the end of G2 due to inhibition of phosphorylation of threonine 14 (Thr14) and tyrosine 15 (Tyr15) of cdk1 [65]. The presence of checkpoint activation induced by DNA damage during G2 prevents activation of the cyclin B/cdk1 complex by the Cdc25C phosphatase, and as a result, mitotic entry is blocked [66]. The checkpoints present in the S and G2/M phases are Chk1 and Chk2, which are Checkpoint Kinases belonging to the serine/threonine protein kinases that are the main regulators of the S and G2/M checkpoints. Chk1 phosphorylates and inhibits cdc25 prevent damaged cells from progressing to the mitotic phase. Inactivating Chk1 allows for uncontrolled cell cycle regulation in the presence of DNA damage, as well as premature mitotic entry, which leads to mitotic failure and cell death [65].

Human polo-like kinase 1 (Plk1) is a kinase with a serine/threonine kinase domain at the amino-terminal and is present in the polo box at the carboxyl-terminal. Polo-like kinase 1 (Plk1) is an important target of DNA damage checkpoints and is a kinase required

for cells to enter the mitotic phase after recovering from DNA damage checkpoints [67]. Inhibition of Plk1 may lead to sensitivity to inhibitors of other pathways, such as androgen signaling inhibitors, thereby preventing cancer tumor growth. Several studies have shown increased levels of Plk1 expression in cancer, which causes cancer cells to overgrow and invade tissues. As a result, inhibiting Plk1 activity in cells causes cell cycle arrest, inhibits tumor growth, and results in cell death, thus allowing for increased human survival [68]. According to Bhui et al. [31], bromelain can inhibit the G2/M phase cell cycle by decreasing the expression of cyclin B1, and Polo-like kinase-1 and increasing myt1 expression.

Author Contributions. The following contributions of each author are confirmed: Diyenda Ainun Nida contributed to the conception and design of the study, data collecting, analysis, and interpretation of the findings, as well as preparation of a draft article. Meanwhile, Haryoto was responsible for reviewing and approving the manuscript.

References

1. F. Y. Ilhami, F. S. Wahyuni, and E. Husni, "Uji Sitotoksik Hasil Fraksinasi Ekstrak Etanol Akar Asam Kandis (Garcinia cowa Roxb.) Terhadap Sel Kanker Payurdara T47D Dengan Metoda MTT," *Pros. Semin. Nas. Perkemb. Terkini Sains Farm. dan Klin. III*, pp. 71–77, 2013.
2. Kemenkes RI, "Hari Kanker Sedunia 2019," *Departemen Kesehatan RI*, pp. 1–4, 2019.
3. R. M. Widyanto, R. N. Halimah, Y. Rahmi, B. Utomo, and W. Diah, "Antioxidant and Cytotoxic Effect of Water Extract of Ananas comosus in Human Breast Cancer Cell Line," *J. Islam. Med.*, vol. 4, no. 2, pp. 123–130, 2020, doi: https://doi.org/10.18860/jim.v4i2.10109.
4. I. A. Rahman, N. Camalxaman, A. S. Rambely, N. Haron, and E. Mohamed, "Ananas comosus (L.) Merr.: A mini review of its therapeutic properties," *Heal. Scope*, vol. 54, no. 2, pp. 54–59, 2020.
5. Umarudin, Y. R. Sari, B. Fal, and Syukrianto, "Efektivitas Daya Hambat Ekstrak Etanol 96% Bonggol Nanas (Ananas Comosus L) Terhadap Pertumbuhan Bakteri Staphylococcus Aureus," *J. Pharm. Sci.*, vol. 3, no. 2, pp. 32–36, 2018.
6. W. Ren, Z. Qiao, H. Wang, L. Zhu, and L. Zhang, "Flavonoids: Promising anticancer agents," *Med. Res. Rev.*, vol. 23, no. 4, pp. 519–534, 2003, doi: https://doi.org/10.1002/med.10033.
7. F. Naritasari, H. Susanto, and Supriatno, "Pengaruh Konsentrasi Ekstrak Etanol Bonggol Nanas (Ananas comosus (L.) Merr) Terhadap Apoptosis Karsinoma Sel Skuamosa Lidah Manusia," *Maj. Obat Tradis.*, vol. 15, no. 1, pp. 16–25, 2010, [Online]. Available: http://journal.ugm.ac.id/TradMedJ/article/view/8064.
8. P. Gupta, I. Bhatnagar, S. K. Kim, A. K. Verma, and A. Sharma, "In-vitro cancer cell cytotoxicity and alpha amylase inhibition effect of seven tropical fruit residues," *Asian Pac. J. Trop. Biomed.*, vol. 4, no. Suppl 2, pp. S665–S671, 2014, doi: https://doi.org/10.12980/APJTB.4.2014B433.
9. M. B. A. Gani, R. Nasiri, J.H. Almaki, F. A. A. Majid, M. Marvibaigi, N. Amini, S. H. Chermahini, M. Mashudin, "In Vitro Antiproliferative Activity of Fresh Pineapple Juices on Ovarian and Colon Cancer Cell Lines," *Int. J. Pept. Res. Ther.*, vol. 21, no. 3, pp. 353–364, 2015, doi: https://doi.org/10.1007/s10989-015-9462-z.
10. M. M. Rashad, A. E. Mahmoud, M. M. Ali, M. U. Nooman, and A. S. Al-Kashef, "Antioxidant and anticancer agents produced from pineapple waste by solid state fermentation," *Int. J. Toxicol. Pharmacol. Res.*, vol. 7, no. 6, pp. 287–296, 2015.

11. N. A. Abdul Halim, S. Ramasamy, B. C. Tan, N. Khalid, and J. S. Yaacob, "In vitro shoot regeneration and analysis of biochemical, antioxidant and anticancer properties of Ananas comosus var. MD2," *Malaysian J. Fundam. Appl. Sci.*, vol. 14, no. 2, pp. 263–268, 2018, doi: https://doi.org/10.11113/mjfas.v14n2.900.
12. M. Khamenkhetkarn, C. Jaikang, T. Sastraruji, and M. Khamenkhetkarn, "Antiproliferative and Antioxidant Properties of Ananas comosus L . Merr ., Patavia and Nanglae , From Northern Thailand," *Sch. Res. Libr.*, vol. 9, no. 11, pp. 38–46, 2017.
13. R. M. Widyanto, J. A. Putri, Y. Rahmi, W. D. Proborini, and B. Utomo, "AKTIVITAS ANTIOKSIDAN DAN SITOTOKSISITAS IN VITRO EKSTRAK METANOL BUAH NANAS (Ananas comosus) PADA SEL KANKER PAYUDARA T-47D," *J. Pangan dan Agroindustri*, vol. 8, no. 2, pp. 95–103, 2020, doi: https://doi.org/10.21776/ub.jpa.2020.008.02.5.
14. T. Tuy-On, A. Itharat, P. Maki, P. Thongdeeying, W. Pipatrattanaseree, and B. Ooraikul, "In Vitro Cytotoxic Activity against Breast, Cervical, and Ovarian Cancer Cells and Flavonoid Content of Plant Ingredients Used in a Selected Thai Traditional Cancer Remedy: Correlation and Hierarchical Cluster Analysis," *Evidence-based Complement. Altern. Med.*, vol. 2020, pp. 1–10, 2020, doi: https://doi.org/10.1155/2020/8884529.
15. Haryoto, Muhtadi, P. Indrayudha, T. Azizah, and A. Suhendi, "Aktivita Sitotoksik Ekstrak Etanol Tumbuhan SALA (Cynometra ramiflora Linn) Terhadap Sel HeLa, T47D dan WiDR," *J. Penelit. Saintek*, vol. 18, no. 21–28, 2013.
16. P. P. Joy, "Benefits and Uses of Pineapple," *Kerala Agric. Univ.*, no. August, 2010, doi: https://doi.org/10.13140/RG.2.1.2782.4888.
17. I. A. Rahman, S. N. Camalxaman, A. S. Rambely, and N. Haron, "Ananas comosus (L .) Merr .: A mini review of its therapeutic properties," pp. 54–59, 2020.
18. J. Azevedo da Paixão *et al.*, "Pharmacological Actions of Ananas comosus L. Merril: Revision of the Works Published from 1966 to 2020," *Pharmacogn. Rev.*, vol. 15, no. 29, pp. 57–64, 2021, doi: https://doi.org/10.5530/phrev.2021.15.6.
19. N. Oktafia, R. Susanti, and N. U. Purwanti, "Uji Toksisitas Akut Ekstrak Etanol Kulit Buah Nanas (Ananas comusus L) Terhadap Tikus Betina (Rattus norvegicus L.) Galur Wistar," *J. Mhs. Farm. Fak. UNTAN*, vol. 4, no. 1, pp. 1–11, 2019.
20. I. A. Reiza, L. Rijai, and F. Mahmudah, "Skrining Fitokimia Ekstrak Etanol Kulit Nanas (Ananas comosus (L.) Merr)," *Proceeding Mulawarman Pharm. Conf.*, vol. 10, pp. 104–108, 2019, doi: https://doi.org/10.25026/mpc.v10i1.371.
21. S. J. Maleki, J. F. Crespo, and B. Cabanillas, "Anti-inflammatory effects of flavonoids," *Food Chem.*, vol. 299, no. July, p. 125124, 2019, doi: https://doi.org/10.1016/j.foodchem.2019.125124.
22. A. Ullah *et al.*, "Importan Flavonoids and Their Role as a Therapeutic Agent," *Molecules*, vol. 25, pp. 1–39, 2020.
23. B. Debnath, W. S. Singh, and K. Manna, "A phytopharmacological review on Ananas comosus," *Adv. Tradit. Med.*, no. 0123456789, 2021, doi: https://doi.org/10.1007/s13596-021-00563-w
24. K. Iwashita, M. Kobori, K. Yamaki, and T. Tsushida, "Flavonoids inhibit cell growth and induce apoptosis in b16 melanoma 4A5 cells," *Biosci. Biotechnol. Biochem.*, vol. 64, no. 9, pp. 1813–1820, 2000, doi: https://doi.org/10.1271/bbb.64.1813.
25. H. W. Zhang *et al.*, "Flavonoids inhibit cell proliferation and induce apoptosis and autophagy through downregulation of PI3Kγ mediated PI3K/AKT/mTOR/p70S6K/ULK signaling pathway in human breast cancer cells," *Sci. Rep.*, vol. 8, no. 1, pp. 1–13, 2018, doi: https://doi.org/10.1038/s41598-018-29308-7.
26. T. Li *et al.*, "Msjor Polyphenolics in Pineapple Peels and Their Antioxidant Interactions," *Int. J. Food Prop.*, vol. 17, pp. 1805–1817, 2014.

27. P. C. Arampath and M. Dekker, "Bulk storage of mango (Mangifera indicaL.) and pineapple (Ananas comosus L.) pulp: effect of pulping and storage temperature on phytochemicals and antioxidant activity," *J. Sci. Food Agric.*, vol. 99, pp. 5157–5167, 2019.
28. A. Müller *et al.*, "Comparative study of antitumor effects of bromelain and papain in human cholangiocarcinoma cell lines," *Int. J. Oncol.*, vol. 48, no. 5, pp. 2025–2034, 2016, doi: https://doi.org/10.3892/ijo.2016.3411.
29. A. A. Al-Hazzani and A. A. Alshatwi, "Catechin hydrate inhibits proliferation and mediates apoptosis of SiHa human cervical cancer cells," *Food Chem. Toxicol.*, vol. 49, no. 12, pp. 3281–3286, 2011, doi: https://doi.org/10.1016/j.fct.2011.09.023.
30. D. M. Kopustinskiene, V. Jakstas, A. Savickas, and J. Bernatoniene, "Flavonoids as anticancer agents," *Nutrients*, vol. 12, no. 2, pp. 1–24, 2020, doi: https://doi.org/10.3390/nu12020457.
31. K. Bhui *et al.*, "Bromelain inhibits nuclear factor kappa-B translocation, driving human epidermoid carcinoma A431 and melanoma A375 cells through G 2/M arrest to apoptosis," *Mol. Carcinog.*, vol. 51, no. 3, pp. 231–243, 2012, doi: https://doi.org/10.1002/mc.20769.
32. A. Amini *et al.*, "Cytotoxic Effect of Bromelain in Human Gastrointestinal Carcinoma Cell Lines (MKN45, KATO-III, HT29-5F12, and HT29-5M21)," *Onco. Targets. Ther.*, vol. 6, pp. 403–409, 2013.
33. B. Romano *et al.*, "The chemopreventive action of bromelain, from pineapple stem (Ananas comosus L.), on colon carcinogenesis is related to antiproliferative and proapoptotic effects," *Mol. Nutr. Food Res.*, vol. 58, no. 3, pp. 457–465, 2014, doi: https://doi.org/10.1002/mnfr.201300345.
34. S. HAIYAN *et al.*, "Growth of Breast Cancer Cells Inhibited by Bromelains Extracted from the Different Tissues of Pineapple," *Folia Biol.*, vol. 68, no. 3, pp. 81–88, 2020, doi: https://doi.org/10.3409/fb_68-3.10.
35. C. M. Pfeffer and A. T. K. Singh, "Apoptosis: A Target for Anticancer Therapi," *Int. J. Mol. Sci.*, vol. 19, no. 448, pp. 1–10, 2018.
36. N. I. Ismail, I. Othman, F. Abas, N. H. Lajis, and R. Naidu, *Mechanism of apoptosis induced by curcumin in colorectal cancer*, vol. 20, no. 10. 2019.
37. F. Cairrao and P. M. Domingos, "Apoptosis: Molecular Mechanism," *Encycl. Life Sci.*, pp. 1–8, 2010.
38. T. J. Fan, L. H. Han, R. S. Cong, and J. Liang, "Caspase family proteases and apoptosis," *Acta Biochim. Biophys. Sin. (Shanghai).*, vol. 37, no. 11, pp. 719–727, 2005, doi: https://doi.org/10.1111/j.1745-7270.2005.00108.x.
39. I. Chowdhury, B. Tharakan, and G. K. Bhat, "Caspases - An update," *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.*, vol. 151, no. 1, pp. 10–27, 2008, doi: https://doi.org/10.1016/j.cbpb.2008.05.010.
40. L. M. Sari, "Cakradonya Dent J; 10(2): 65-70," *Cakradonya Dent. J.*, vol. 10, no. 2, pp. 65–70, 2020.
41. S. Goldar, M. S. Khaniani, S. M. Derakhshan, and B. Baradaran, "Review: Molecular Mechanisms of Apoptosis and Roles in Cancer Development and Treatment," *Asian Pacific J. Cancer Prev.*, vol. 16, no. 6, pp. 2129–2144, 2015.
42. A. Ashkenazi, "Targeting the extrinsic apoptosis pathway in cancer," *Cytokine Growth Factor Rev.*, vol. 19, no. 3–4, pp. 325–331, 2008, doi: https://doi.org/10.1016/j.cytogfr.2008.04.001.
43. R. S. Y. Wong, "Apoptosis in cancer: From pathogenesis to treatment," *J. Exp. Clin. Cancer Res.*, vol. 30, no. 1, pp. 1–14, 2011, doi: https://doi.org/10.1186/1756-9966-30-87.
44. S. Zaman, R. Wang, and V. Gandhi, "Targeting the apoptosis pathway in hematologic malignancies," *Leuk. Lymphoma Inf. Healthc.*, vol. 55, no. 9, pp. 1980–1992, 2014, doi: https://doi.org/10.3109/10428194.2013.855307.
45. J. C. Reed, "Warner-Lambert/Parke Davis award lecture: Mechanisms of apoptosis," *Am. J. Pathol.*, vol. 157, no. 5, pp. 1415–1430, 2000, doi: https://doi.org/10.1016/s0002-9440(10)64779-7.

46. R. Jan and G.-1-saba Chaudhry, "Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics," *Adv Pharm Bull*, vol. 9, no. 2, pp. 205–218, 2019.
47. K. Bhui, S. Prasad, J. George, and Y. Shukla, "Bromelain inhibits COX-2 expression by blocking the activation of MAPK regulated NF-kappa B against skin tumor-initiation triggering mitochondrial death pathway," *Cancer Lett.*, vol. 282, no. 2, pp. 167–176, 2009, doi: https://doi.org/10.1016/j.canlet.2009.03.003.
48. A. Greenhough *et al.*, "The COX-2/PGE2 pathway: Key roles in the hallmarks of cancer and adaptation to the tumour microenvironment," *Carcinogenesis*, vol. 30, no. 3, pp. 377–386, 2009, doi: https://doi.org/10.1093/carcin/bgp014.
49. A. V. Vaseva and U. M. Moll, "The mitochondrial p53 pathway," *Biochim. Biophys. Acta - Bioenerg.*, vol. 1787, no. 5, pp. 414–420, 2009, doi: https://doi.org/10.1016/j.bbabio.2008.10.005.
50. M. Burotto, V. L. Chiou, J. M. Lee, and E. C. Kohn, "The MAPK pathway across different malignancies: A new perspective," *Cancer*, vol. 120, no. 22, pp. 3446–3456, 2014, doi: https://doi.org/10.1002/cncr.28864.
51. V. Grossi, A. Peserico, T. Tezil, and C. Simone, "p38α MAPK pathway: A key factor in colorectal cancer therapy and chemoresistance," *World J. Gastroenterol.*, vol. 20, no. 29, pp. 9744–9758, 2014.
52. C. Braicu *et al.*, "Review: A Comprehensive Review on MAPK: A PromisingTherapeutic Target in Cancer," *Cancers (Basel).*, vol. 11, pp. 1–25, 2019.
53. N. Ghosh, R. Chaki, V. Mandal, and S. C. Mandal, "COX-2 as a target for cancer chemotherapy," *Pharmacol. Reports*, vol. 62, pp. 233–244, 2010.
54. N. Kalra *et al.*, "Regulation of p53, nuclear factor κB and cyclooxygenase-2 expression by bromelain through targeting mitogen-activated protein kinase pathway in mouse skin," *Toxicol. Appl. Pharmacol.*, vol. 226, no. 1, pp. 30–37, 2008, doi: https://doi.org/10.1016/j.taap.2007.08.012.
55. F. Chang *et al.*, "Invovement of P13K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy," *Mol. Targets Ther.*, vol. 17, pp. 590–603, 2003.
56. M. Osaki, M. Oshimura, and H. Ito, "PI3K-Akt pathway: Its functions and alterations in human cancer," *Apoptosis*, vol. 9, no. 6, pp. 667–676, 2004, doi: https://doi.org/10.1023/B:APPT.0000045801.15585.dd.
57. B. Jingwen, L. Yaochen, and Z. Guojun, "Cell cycle regulation and anticancer drug discovery," *Cancer Biol. Med.*, vol. 14, no. 4, p. 348, 2017, doi: https://doi.org/10.20892/j.issn.2095-3941.2017.0033.
58. H. Murti, A. Boediono, B. Setiawan, and F. Sandra, "Regulasi Siklus Sel : Kunci Sukses Somatic Cell Nuclear Transfer," *Cdk*, vol. 34, no. 6, pp. 312–316, 2007.
59. Y. Sun, Y. Liu, X. Ma, and H. Hu, "The influence of cell cycle regulation on chemotherapy," *Int. J. Mol. Sci.*, vol. 22, no. 13, pp. 1–25, 2021, doi: https://doi.org/10.3390/ijms22136923.
60. T. Otto and P. Sicinski, "Cell cycle proteins as promising targets in cancer therapy," *Nat. Rev. Cancer*, vol. 17, no. 2, pp. 93–115, 2017, doi: https://doi.org/10.1038/nrc.2016.138.
61. S. Lim and P. Kaldis, "Cdks, cyclins and CKIs: Roles beyond cell cycle regulation," *Dev.*, vol. 140, no. 15, pp. 3079–3093, 2013, doi: https://doi.org/10.1242/dev.091744.
62. S. Diaz-Moralli, M. Tarrado-Castellarnau, A. Miranda, and M. Cascante, "Targeting cell cycle regulation in cancer therapy," *Pharmacol. Ther.*, vol. 138, no. 2, pp. 255–271, 2013, doi: https://doi.org/10.1016/j.pharmthera.2013.01.011.
63. T. D. Pollard, J. Lippincott-Schwartz, W. C. Earnshaw, and G. T.Johnson, Eds., "G2 Phase, Responses to DNA Damage, and Control of Entry Into Mitosis," in *Cell Biology*, Third Edit., 2017, pp. 743–754.
64. J. Chow and R. Poon, "The CDK1 inhibitory kinasi MYT1 in DNA damage checkpoint recovery," *Oncogene*, vol. 32, pp. 4778–4788, 2013.

65. W. Xu and G. McArthur, "Cell Cycle Regulation and Melanoma," *Curr. Oncol. Rep.*, vol. 18, no. 6, 2016, doi: https://doi.org/10.1007/s11912-016-0524-y.
66. V. A. J. Smits, R. Klompmaker, L. Arnaud, G. Rijksen, E. A. Nigg, and R. H. Medema, "Polo-like kinase-1 is a target of the DNA damage checkpoint," *Nat. Cell Biol.*, vol. 2, no. 9, pp. 672–676, 2000, doi: https://doi.org/10.1038/35023629.
67. V. Lobjois, D. Jullien, J. P. Bouché, and B. Ducommun, "The polo-like kinase 1 regulates CDC25B-dependent mitosis entry," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1793, no. 3, pp. 462–468, 2009, doi: https://doi.org/10.1016/j.bbamcr.2008.12.015.
68. E. G. Colicino and H. Hehnly, "Regulating a key mitotic regulator, polo-like kinase 1 (PLK1)," *Cytoskeleton*, vol. 75, no. 11, pp. 481–494, 2018, doi: https://doi.org/10.1002/cm.21504.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits any noncommercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.