REVIEW: POTENTIAL ACA (1-ACETOXYCHAVICOL ACETATE) FROM *Alpinia galanga* RHIZOME AS AN ANTICANCER

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ABSTRACT:
Background: *Cancer is a group of diseases characterized by abnormal cell growth and is one of the leading causes of death worldwide. Alpinia galanga is a rhizome that is widely used as a spice in cooking and traditional medicine in various countries. 1'-acetoxychavicol acetate (ACA) is the main component found in the rhizome of Alpinia galanga.*
Objective: *To review the components of ACA which exhibit anticancer activity in several cancer cell lines.*
Materials and Methods: *In compiling this review article, the technique used is to use literature studies by searching for sources or literature in the form of primary data or the form of official books and international journals in the last 10 years. Search for the main references used in this review article through trusted webs such as ScienceDirect, PubMed, NCBI, ResearchGate, and Google Scholar which focus on the active compounds and anticancer effects of Alpinia galanga.*
Results: *The studies published from 2010-17 were selected. Types of cancer and cell lines that can be inhibited are breast cancer, cervical cancer, lung cancer, colorectal cancer, neck and head cancer, liver cancer, and prostate cancer.*
Conclusion: *Galangal (Alpinia galanga) is a medicinal plant that has many benefits. 1'-acetoxychavicol acetate (ACA) is the main component found in the rhizome of Alpinia galanga which has potential as an anticancer. The results of research that has been conducted in recent years have shown the potential of ACA in inhibiting the growth of cancer cells. The potential of ACA as an anticancer can be seen from the IC_{50} value and through several mechanisms, namely inhibition of proliferation, inhibition of angiogenesis, and induction or increase in apoptosis.*

Keywords: Alpinia galanga**,** 1'-acetoxychavicol acetate (ACA), traditional uses, anticancer effect

INTRODUCTION:

Cancer is a group of diseases characterized by uncontrolled growth in the regeneration process so that cells grow abnormally [1]. Cancer is one of the leading causes of death worldwide. By 2020, 1,806,590 new cancer cases and 606,520 cancer deaths are projected to occur in the United States [2].

The most common types of cancer are breast cancer, lung, and bronchial cancer, prostate cancer, colon and rectum cancer, skin melanoma, bladder cancer, non-Hodgkin's lymphoma, kidney and renal pelvic cancer, pelvic cancer, endometrial cancer, leukemia, cancer. pancreas, thyroid cancer, and liver cancer [3].

Medicinal plants have been used since ancient times for various kinds of treatment diseases including cancer. The enormous diversity of plants is a source that is very potential from natural materials / chemical compounds that have anti-tumor activity and cytotoxic [4]. Anticancer from herbal plants can be extracts or single active compounds isolated from plants [5]. One type of plant that has anticancer activity is *Alpinia galanga*. Galangal, Laos, or Kelawas (Karo) is a type of tuber plant that can live in the highlands and lowlands. Generally, people use it as a mixture of cooking spices [6]. *A.galanga* which is known as a spice plant in Asian countries is also widely used as a herbal medicine for various diseases [7]. The potential uses for this herbal medicine have been explored, including the findings of its active ingredient [8].

One of the phenylpropanoid compounds from *A.galanga* is 1'-acetoxychavicol acetate (ACA). These compounds have shown various pharmacological effects such as anticancer effects, gastroprotective effects, xenobiotic protection, allergy activity, antimicrobial activity, and anti-dementia effects [9]. ACA is one of the compounds reported to exhibit potent anticancer effects against various cancer cells, such as colorectal adenocarcinoma cells, cervical cancer cells, oral squamous carcinoma cells, prostate cancer cells, breast cancer cells, glioblastoma cells, myeloma cells, hepatocellular carcinoma cells, and Ehrlich ascitic tumor cells (EATCs) [9, 10].

MATERIALS AND METHODS:

Reporting: All complete studies conducted on ACA's anticancer activity are available for free on the internet.

Inclusion and Exclusion Criteria: All complete original articles are included in this review. The study period 2010-2017 was selected. Studies that report activity in inhibiting cancer cells are included. Studies that have irrelevant titles, objectives, methods, or statistical tests are excluded. All studies that had incomplete or confusing content, methods, references, and author information are also excluded. To ensure completeness of the desired attributes, a checklist was created containing cancer cell types, cell lines, and comments on the effect of ACA.

Search Strategy and Information Sources: In compiling this review article, the technique used is to use literature studies by searching for sources or literature in the form of primary data or the form of official books and international journals in the last 10 years. Search for the main references used in this review article through trusted webs such as ScienceDirect, PubMed, NCBI, ResearchGate, and Google Scholar which focus on the active compounds and anticancer effects of *Alpinia galanga*.

Study Selection: In the first step, the study was taken in a reference management software named MENDELEY for storage and avoiding duplication. The studies taken are then

assessed via the checklist mentioned above. Irrelevant or ambiguous studies were excluded. In the second step, the author critically analyzes the content of the article. Articles that did not match the title had irrelevant variables, or analyzes that were incorrectly excluded were excluded.

Data Extraction: A structured data extraction form was created to extract information from selected studies. the first type of cancer, cell lines, comments on the effects of ACA, and references. The two reviewers independently extracted data from the articles. Any discrepancies in the reported data were reviewed and corrected by a third reviewer.

Alpinia galanga is an important medicinal plant in many traditional medicine systems for treating several diseases, including microbial infections, inflammation, rheumatism, chest pain, dyspepsia, fever, heartburn, kidney disease, tumors, diabetes, and even HIV[11]. In the Ayurvedic system, *A.galanga* is used to increase appetite, taste, and sound. Also to treat bronchitis and heart disease [12]. In the Unani system, rhizomes have been used as a medicine for stomach aches, aphrodisiac, tonic, diuretic, expectorant, carminative, useful for headaches, rheumatism, laryngitis, gastric erosion, chest pain, diabetes, heartburn, tubercular glands, and diseases kidney. In the Thai system of medicine, this rhizome is widely used as a carminative, antiflatulent, anti-fungal, and anti-itch [13]

Alpinia galanga is grouped in the Zingiberaceae family, has active compounds such as 1-acetoxychavicol acetate (ACA), acetoxyeugenol acetate (AEA), hydroxychavicol acetate (HCA), trans-phydroxycinnamaldehyde, flavonoids (kaemperol, kaempferida, galangin) [12, 14]. 1'-acetoxychavicol acetate (ACA) isolated from Alpinia galanga is the main compound that has various biological activities.[15]

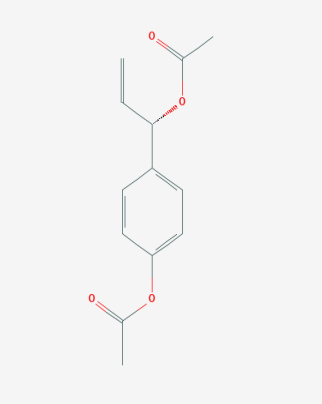

Figure 1. 2D Structure of 1'Acetoxychavicol acetate.[16]

ACA performs its biological activity by forming quinone methide intermediates, as shown in Figure 2. The formation of quinone methide intermediates is generally associated with ester hydrolysis at position [4], followed by the elimination of the acetoxy group at position [10]. The methoxy group is not only important for anticancer activity but position. It is also important in the effectiveness of inhibition of cancer cell growth compounds.[17].

Toxicity is a measure of any unwanted or side effect of a chemical (drug). This specific type of adverse effect is called

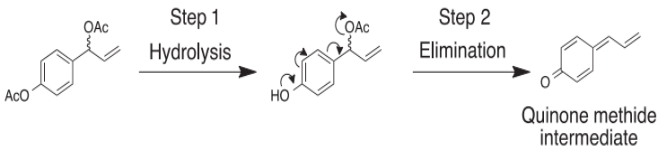

Figure 2. The mechanisms assumed to underlie the biological action of ACA.[18]

endpoint toxicity as well as carcinogenicity or genotoxicity in the form of quantitative (example: LD50: 50% lethal dose tested on each individual) while for qualitative as a pair (example: toxic - non-toxic) or as usual (example: low, moderate, or high toxicity).[19] Toxicity studies of intravenous ACA administration in Sprague-Dawley rats, acute (single dose 2.00, 4.00 and 6.66 mg/kg, for 14 days), and sub-acute (with weekly injections of 0.66, 1, 33, and 2.22 mg/kg, for 28 days) there was no change in behavior or bodyweight, nor did they cause any changes in

RESULTS:

In the development of new anticancer drugs as candidates for cancer therapy agents, preclinical evaluation is one of the important things to determine the potential for its cytotoxic activity. Evaluation that has been standardized to determine whether a material contains a biologically toxic material is called a cytotoxic test [21].

Cytotoxic test is used as an initial stage to determine the effect of a natural substance in inhibiting tumor cell growth. A compound is considered active if it can inhibit the growth of 50% of the tumor cell population at a certain concentration. One of the methods commonly used to assess this cytotoxic activity is the MTT method [22.23]. The cytotoxicity test parameter was the IC_{50} value. IC_{50} is a concentration that can cause living cells as much as 50% of the cancer cell population and the rest is expected to die. The smaller the IC_{50} value, the higher the hematological parameters with an LD50 value of 6.66 mg/kg BW[20]. The potential of the test compound as a cytotoxic agent is defined as a compound to have cytotoxic activity if it can inhibit the growth of 50% of the cell population at a concentration below 200 µg / ml (IC_{50}: 200 µg / ml).[22,23,24]. The potential of a compound is classified as a strong cytotoxic agent if the IC_{50} < 20µg. / mL, moderate if the IC_{50} < 50 µg / mL, and weak if the IC_{50} > 50 µg / mL.[25]

Research using ACA in vitro has provided results indicating its role in several cancer cells (Table 1). Researchers reported that *A. galanga* with the highest ACA concentration showed very strong cytotoxic activity, with IC_{50} values of 12.50, 15.80, and 13.20 µg / ml against T47D and MCF7 breast cancer cells, as well as cervical cancer HeLa cells, respectively [26]. ACA, which is the main cytotoxic component, also showed significant cytotoxic activity against COR L23 lung cancer cells and MCF7 breast cancer cells with an IC_{50} of 7.8 µ M and 23.9 µM [12]. According to Zeng et al, The ACA component in *A.galanga* also showed significant cytotoxicity against HeLa, A549, HepG2, and SMMC-7721 cell line with IC_{50}, 85.1, 64.44, 74.51, and 61.27 µg / ml [27] Liew *et al*, also reported IC_{50} values against MDa-MB-231 cells < 30.0 µg / ml [28].

Table 2: ACA Anticancer activity

No	Cancer type	Cell line	Comment on the effects of ACA / IC_{50} (µg/ml)	Reference
1	Breast Cancer	T47D	IC_{50} = 12.50	[26]
		MCF7	IC_{50} = 15.80	[26]
		MCF7	IC_{50} = 23.9	[12]
		MDa-MB-231	IC_{50} = < 30.0	[28]
2	Cervical Cancer	HeLa	IC_{50} = 13.20 ; IC_{50} = 85.1	[26,27]
		CaSki	Decreases miRNA-210 expression	[40]
		SiHa	Increase SMAD4 homologous expression	[40]
3	Lung Cancer	COR L23	IC_{50} = 7.8	[12]
		A549	IC_{50} = 64.44	[27]
4	Colorectal Cancer	SW480	IC_{50} = 80 µM Inhibits proliferation, increases apoptosis, increases expression of p21	[15]
5	Head and Neck Cancer	HN4	Increase apoptosis, decrease miRNA-23a expression	[37]
6	Liver Cancer	HepG2	IC_{50} = 74.51	[27]
		SMMC-7721	IC_{50} = 61.27	[27]
7	Prostate Cancer	PC-3	Inhibits proliferation, tubulogenesis, and decreases the expression of VEGF and Ki-67	[43]

DISCUSSION:

In addition to seeing the cytotoxic parameters of the IC_{50} value, various types of cancer cells can also be inhibited growth through cell proliferation, apoptosis, and angiogenesis.

1. Inhibition of Cancer Cell Proliferation

Cells that are in the proliferation phase express several specific proteins that can be detected with certain monoclonal antibodies. Cell proliferation activity can be detected using immunohistochemical Ki-67 which will express each phase of the cell cycle [29]. Ki-67 is a cell nucleus antigen that is in the G1, S, G2, and M phases of the cell cycle, so it can be used as a marker of proliferation [30]. ACA can inhibit the proliferation of colon cancer cells SW480, the cell cycle is stopped in the G0/G1 phase with considerable DNA damage and mitochondrial depolarization [15].

2. Increased Apoptosis

Apoptosis is programmed cell death [31]. Apoptosis is a process by which cells stop growing and dividing which ultimately results in controlled cell death without spilling its contents into the surrounding environment.[32]

Cancer cell death can occur with the activation of the apoptotic pathway characterized by caspase activation.[33] Caspase-3 belongs to the caspase executor group which is activated by the caspase initiator, for example, caspase-8 and caspase-9. Activation of apoptosis in both extrinsic and intrinsic pathways will lead to activation of caspase-3 as the executor caspase. When caspase-3 has been activated, cell death will occur in the form of apoptosis [34]. ACA has anticancer activity by induction of apoptosis through the mechanism of activation of the caspase-3 pathway in C3H mouse adenocarcinoma cells [35].

A study conducted by Baradwaj *et al*, Showed that ACA can increase apoptosis by increasing p21 expression in colon cancer. [15] p21 is a protein suppressor tumor weighing 21 KDa and has a major function in the regulation of cell cycle progression [36].

ACA induces apoptosis and inhibit the growth of HN4 cells in human head and neck squamous carcinoma (HN4) by downregulating miRNA-23a expression [37]. miRNA is a highly conserved uncoded small RNA that regulates the expression of post-transcription genes that can be found in tissues and blood circulation [38]. A single miRNA is capable of regulating multiple genes, a gene can be targeted by different miRNAs, and each miRNA can regulate human tumorigenesis, cell proliferation, metabolism, or apoptosis [39]. ACA can also decrease miRNA-210 expression in cervical cancer cells (CaSki) and increase SMAD4 homologous expression [40].

3. Inhibition of Angiogenesis

Angiogenesis is the process of forming new blood vessels that occur normally and is very important in the process of growth and development. The process of angiogenesis is an indicator of a change in the status of cancer cells from benign to malignant. Cancer cells are known to be maglina if they measure more than 2 mm3 [41]. Among the many angiogenic factors identified, Vascular Endothelial Growth Factor (VEGF) is the most powerful endothelial cell-specific mitogen that plays a key role in the complex process of angiogenesis. VEGF is the main regulator of abnormal

angiogenesis it works with stimulates mitogenesis of endothelial cells and increases vascular permeability [42].

As research conducted by Pang *et al*, that ACA leads to angiogenesis-mediated prostate tumor suppression. They also stated that ACA can decrease VEGF expression, which is to inhibit migration induced by vascular endothelial growth factors, adhesion and tubulogenesis of human umbilical vascular endothelial cells [43].

CONCLUSION:

Galangal (*Alpinia galanga*) is a medicinal plant that has many benefits. 1'-acetoxychavicol acetate (ACA) is the main component found in the rhizome of Alpinia galanga which has potential as an anticancer. The results of research that has been conducted in recent years have shown the potential of ACA in inhibiting the growth of cancer cells. The potential of ACA as an anticancer can be seen from the IC_{50} value and through several mechanisms, namely inhibition of proliferation, inhibition of angiogenesis, and induction or increase in apoptosis.

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