

Identification of Anticancer Active Compound from GC-MS Test Results of Zodia Leaves (*Evodia suaveolens*) Ethanol Extract

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ABSTRACT

Cancer is estimated to be the second leading cause of death globally in 2018. The background of the study was the prevalence of cancer in Indonesia in 2018 reaching 775.120 people. Treatments such as surgery, chemotherapy, radiotherapy, immunotherapy, and hormone therapy have negative side effects. Herbal medicine is an alternative treatment with lower side effects that can reduce the side effects of existing treatments. The aim of this study was to identify the results of GC-MS active compounds from ethanol extract of zodia leaves which have the potential as anticancer. The method of this research was observational research through scientific literature. GC-MS results showed 22 active compounds contained in ethanol extract of zodia leaves. Based on the literature searching, there are 17 active compounds that have potential to be anticancer. Menthofuran; evodone; Bicyclopentene; Delta-Selinene; 3,5,7-Octatrien-2-Ol, 2,6-Dimethyl; Aromadendrene oxide 2; Alpha-bisabolol; 2,4-Dimethyl-2,4-Heptadienal; Phytol; Squalene; Beta-Tocopherol; D-alpha-Tocopherol; Stigmasta-5,23-dien-3.beta.-ol; (23S)-ethylcholest-5-en-3.beta.ol; 24,25-Dihydrolanosterol; Lanosterol; and Obtusifoliol. The compounds are targeted in breast, lung, and ovarian cancer.

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Introduction

Cancer is a disease that characterized by excessive cell growth can attack adjacent body parts or spread to other organs. Based on the WHO (World Health Organization) cancer is estimated to be the second leading cause globally in 2018 (Organization, 2019). The prevalence of cancer in Indonesia in 2018 reached 775,120 people with mortality reaching 207,210 of 348,809 cases.

Some approaches used to treat cancer are surgery, chemotherapy, radiotherapy, immunotherapy, targeted therapy, and hormone therapy (Health, 2019). Therapy can be given separately or in combination as adjuvant or neoadjuvant depending on the stage of the cancer (Nurhayati & Lusiyanti, 2006; Sari, Wahid, & Suchitra, 2019). Surgery and radiotherapy treatment are effective for early cancer, but not for metastatic cancer. Chemotherapy, targeted

therapy, and hormone therapy are more appropriate for metastatic cancer (Chabner & Roberts, 2005). But these treatments cause side effects, chemotherapy can affect normal cells and some drugs cannot be combined because it can strengthen the work of other drugs that can increase their side effects to cause death (Lander et al., 2001; Sari et al., 2019). Radiotherapy can affect normal cells causing xerostomia, central nerve complications, and RIHD (Radiation Induced Heart Disease) (Fitriatuzzakiyyah, Sinuraya, & Puspitasari, 2017). To reduce these side effects it is necessary to find anticancer agents from natural products to prevent and cure cancer.

Herbal medicine is an alternative therapy because it is considered to have lower side effects and can even reduce the side effects of chemotherapy and radiotherapy (Radji, Aldiarti, Harahap, & Irawan, 2010). Based on the Minister of Health Decree in 2007, Indonesia has 30,000 plant species which 300 species have been used as traditional medicinal materials from 9,600 species as medicinal plants. Some plants that are used as anticancer include crown god (*Phaleria macrocarpa*), turmeric (*Curcuma longa*), white turmeric (*Curcuma zedoaria*), kopasanda (*Chromolaena odorata* L.) and red fruit (*Pandanus conoideus*) (Fitrah, Winarno, & Simanjuntak, 2017; Lisdawati, 2009; Mutiah, 2015; Radji et al., 2010). Research related to curcumin activity as an anticancer has been widely carried out both in vivo, in vitro, until clinical studies. Based on research that has been done, the administration of curcumin monotherapy can reduce the volume of bladder cancer using human bladder cancer on orthotopic mouse models. In silico and in vivo research shows that curcumin plays a role in inhibiting cell apoptosis and regulating transcription factors (Mutiah, 2015).

Indonesian endemic plants that have the potential to be anticancer namely zodia (*Evodia suaveolens*) which is commonly found as ornamental plants in Java (Kusnoto, 2007). Zodia is often used as an anti-mosquito by rubbing it directly on the body, formed as soap, and spray (Muhamat, Wahyuni, Rusmiati, & Jumar, 2016; Simaremare, Sinaga, & Agustini, 2017). Studies that have been done stated that the content of evodiamine in Zodia (*Evodia sauveolens*) has been shown to have anticancer activities by inhibiting proliferation, invasion, and metastasis, and inducing apoptosis in various types of tumor cells (Jiang & Hu, 2009).

Testing of plant compounds as potential drugs can be done in vivo and in vitro, but this method requires a lot of cost and time, so it is necessary to first examine the interaction of compounds with receptors while eliminating compounds that have low activity through the in silico test (Ekins, Mestres, & Testa, 2007; Ruswanto, 2015). Untuk mengurangi kekurangan tersebut dapat diatasi dengan menggunakan uji secara komputasi atau *in silico*. The computational testing or in silico can reduce these deficiencies and could be done by many methods, one of which is molecular docking. Molecular docking is a computational procedure that describes interactions between drug molecules as ligands and protein receptors (Suhud, 2015). Before conducting the in silico test, it is necessary to identify the active compound contained in a sample to be tested.

There are many methods that can be used to identify active compounds contained in a sample. One of method used is Gas Chromatography - Mass Spectrum (GC-MS), a chromatographic technique to detect volatile compounds and identify molecular weights and molecular formulas (Darmapatni, Basori, & Suaniti, 2016). This

study aims to identify and determine the active compound from ethanol extract of zodia leaves from GC-MS analysis which has the potential as an anticancer.

Material and Methode

This research was conducted in April 2019 at the Physiology Laboratory of the Biology Department of the Faculty of Mathematics and Natural Sciences, University State of Surabaya. The tools used in this study include blenders, rotary evaporators, and GC-MS tools. The ingredients used are zodia leaves, 96% ethanol, and filter paper. The steps taken are:

1. Sampling

The zodia (*Evodia suaveolens*) leaves collection was obtained from several locations of gardens and yards in the Surabaya that cultivated the plants to obtain sufficient quantities for extraction.

2. Extraction Zodia Leaves (*Evodia suaveolens*)

Zodia leaves are washed and then dried for three days and avoided from the sun. The leaves are dried using an oven for two to three days at 60°C, then mashed with a blender until it becomes powder and sifted using a 40 mesh sieve. The zodia leaves powder macerated using 96% polar solvent for 3 days with the concentration of the solvent compared to the first day was 1 : 3 (weight : volume) while the concentration on the second and third days was 1: 1 (weight : volume). The results of maceration were evaporated using a rotary evaporator to form a paste. The extraction results are 100% extract of zodia leaf.

3. Identification Active Compound

The identification of active compounds contained in zodia leaf extract will be carried out using GC-MS analysis. Analysis using the AGILENT 6980N Network GC System with an autosampler. The sample is heated at a temperature of

50-280 °C with an increase in temperature of 100 °C / minute and leave it for 10 minutes. Helium gas is used as a carrier with a flow of 1.3 ml / minute. Heat the injector at 280 °C, the size of the injection of 1µl neat, with a split ratio of 1:10. The interface and source of the MS ion are maintained at 230 °C and 150 °C carefully. The mass spectrum is obtained at 70 eV and in the reading range 200 - 700 amu. The data obtained was analyzed in the GC-MS solution software. This stage was carried out in the Testing Service Unit (ULP), Faculty of Pharmacy, Airlangga University Surabaya.

Results and Discussions

This research was a descriptive experimental study by identifying potentially anticancer compounds from the results of GC-MS test ethanol extract of zodia leaves (figure 1).

From the GC-MS test results, 22 names of compounds were identified through the *pubchem.ncbi.nlm.nih.gov* database (Table 1). Of the 22 compounds there are 17 compounds that have the potential to be anticancer. Based on web server *swisstargetprediction.ch* analysis, menthofuran; evodone; Bicyclopentene; Delta-Selinene; 3,5,7-Octatrien-2-Ol, 2,6-Dimethyl-; aromadendrene oxide 2; Alpha-bisabolol; 2,4-Dimethyl-2,4-Heptadienal; Phytol; Squalene; (23S)-ethylcholest-5-en-3.beta.ol; 24,25-Dihydrolanosterol; Lanosterol; dan Obtusifoliol compound are potential as breast anticancer targeted at CYP450 gene. In breast cancer, one of the genes that is targeted by these compounds is CYP19A1 which is a gene that encodes the aromatase enzyme (Hamid, Sugiyanto, Meiyanto, & Widayarni, 2009). This enzyme role is to catalyze C19 androgens become C18 estrogen. Estrogen and estrogen receptor complexes will stimulate the growth of breast epithelial tissue and

have a local effect. Polymorphism in one of the enzyme coding genes or estrogen receptors will cause changes in milieu of the breast and potentially cause malignancy (Crandall et al., 2009).

Evodone has the potential as an anticancer ovary through its interaction with the influential 3HBM protein on the PI3KCA pathway. This pathway is one of the signal transduction pathways that plays

a role in cell proliferation and differentiation. In cancer cells, this pathway experiences hyperactivation resulting in excessive activation of protein kinase B (PKB) (Brown & Toker, 2015). Excessive activity of PKB causes inhibition of pro-apoptotic protein activation, activation of anti-apoptotic proteins, and increased metastasis (McDonald, 2008).

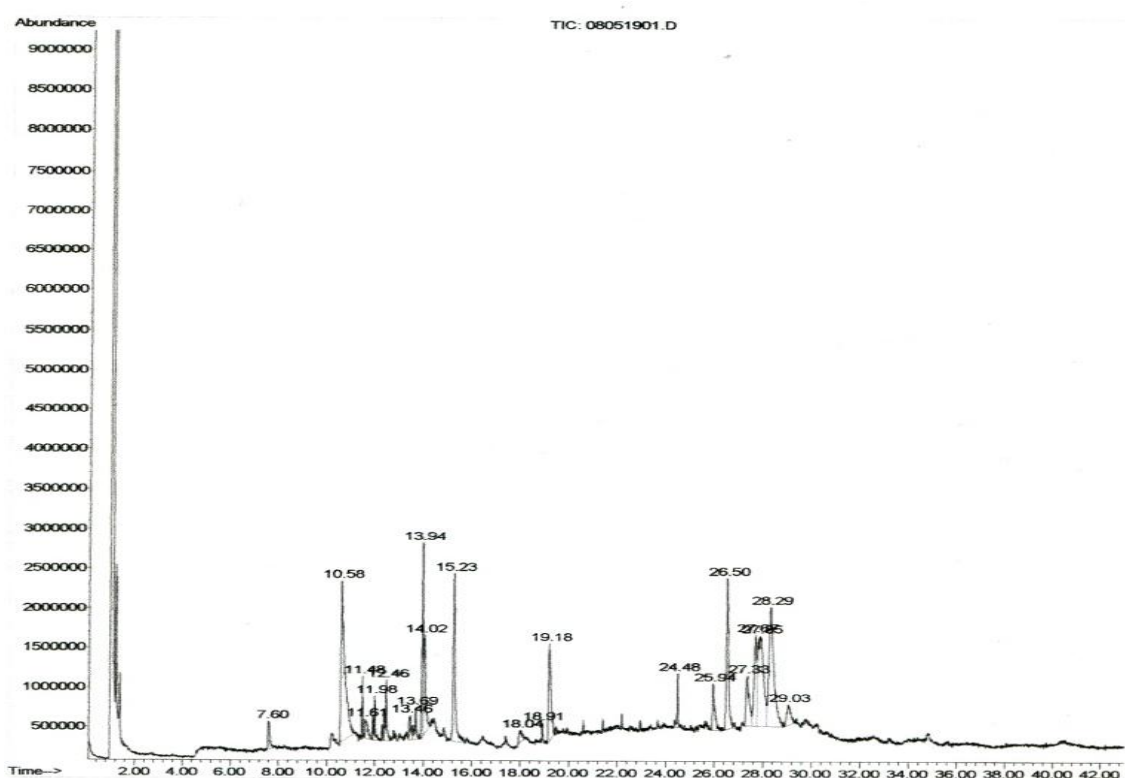


Figure 1. GC-MS Test Results Ethanol Extract of Zodia Leaves

Table 1. Compound Identification Results

Peak	Retention time (minute)	Komposisi (%)	The name of compound
1	7,6	1,24	Menthofuran
2	10,59	14,74	Evodone
3	11,48	0,81	Caryophyllene
4	11,61	1,42	1,1'-Bicyclopentene
5	11,98	1,32	Alpha-Gurjenene
6	12,46	2,14	Delta-Selinene
7	13,46	1,17	3,5,7-Octatrien-2-Ol, 2,6-Dimethyl-
8	13,69	1,33	Aromadendrene oxide 2
9	13,94	5,04	Alpha-bisabolol
10	14,02	4,74	Gamma-Selinene
11	15,23	8,04	2,4-Dimethyl-2,4-Heptadienal

Peak	Retention time (minute)	Komposisi (%)	The name of compound
12	18,04	0,77	-
13	18,91	0,51	Bicyclo[2.2.1]heptane, 2-ethenyl-2, 4-bishomobrendan-7-one
14	19,18	5,76	Phytol
15	24,49	1,31	Squalene
16	25,95	2,76	Beta-Tocopherol
17	26,5	7,79	D-alpha-Tocopherol
18	27,33	3,94	Stigmasta-5,23-dien-3.beta.-ol
19	27,67	5,8	(23S)-ethylcholest-5-en-3.beta.ol
20	27,85	12,82	24,25-Dihydrolanosterol
21	28,28	16,55	Lanosterol
22	29,03	16,55	Obtusifoliol

Compounds that have the potential to be anticancer lungs, namely evodone; 3,5,7-Octatrien-2-Ol, 2,6-Dimethyl; 2,4-Dimethyl-2,4-Heptadienal; Phytol; and Squalene with protein targets namely EGFR, CCNE1, and CYP450. EGFR (Epidermal Growth Factor Receptor) is a 170-kDa glycoprotein protein tyrosine kinase which is present in cell membranes. In normal cells EGFR expression includes epidermal, mesenchymal, and neurogenic cells. EGFR expression when binding to EGF activates cell proliferation and differentiation. DNA mutations in EGFR as detected by polymerase chain reaction (PCR) can occur in the appropriate area with parts of extracellular or intracellular proteins. In non-small cell lung cancer, overexpression of EGFR or mutations in intracellular EGFR (Bethune, Bethune, Ridgway, & Xu, 2010; Inamura, Ninomiya, Ishikawa, & Matsubara, 2010).

Based on searching through a Swiss Target webserver, it can be seen that the compounds of Beta-Tocopherol and Stigmasta-5,23-dien-3.beta.-ol have potential to be lung anticancer and colorectal by targeting epidermal growth factor erbB1 (EGFR) which is on protein kinase. The EGFR gene starts when the ligand is bound to the outermost part of the EGFR receptor protein so that another

homodimer or heterodimer structure will be formed. The results of the formation of this structure will activate the receptor kinase and autophosphorylate specific tyrosine residues that have the ends of the -COOH group. The autophosphorylation process will activate the signal transduction pathways that are regulated by EGFR such as the AKT and ERK pathways that control several cellular processes (Ladanyi & Pao, 2008).

Conclusion

Based on research that has been done, it can be seen that the active compounds contained in the ethanol extract of zodia leaves which have the potential as anticancer, there are Menthofuran; evodone; Bicyclopentene; Delta-Selinene; 3,5,7-Octatrien-2-Ol, 2,6-Dimethyl; Aromadendrene oxide 2; Alpha-bisabolol; 2,4-Dimethyl-2,4-Heptadienal; Phytol; Squalene; Beta-Tocopherol; D-alpha-Tocopherol; Stigmasta-5,23-dien-3.beta.-ol; (23S)-ethylcholest-5-en-3.beta.ol; 24,25-Dihydrolanosterol; Lanosterol; and Obtusifoliol. The compounds are targeted in breast, lung, and ovarian cancer.

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