Isolation of Active Compound from Zingiber Purpureum Roxb. Using Bioassay Guided

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Zingiber purpureum Roxb. was a plant that has th tential to be developed as a chemotherapy agent. Bioassay guided fractination aims to examine the fractions from of bar e plant rhizome to colon cancer cell type WiDr and Vero cells. The apoptotic test and modulating by Isolation process of most active fraction. The pure isolates obtained were identified by structures with structural elucidat using ultraviolet, 1D-NMR, 2D-NMR and liquid chromatography-mass spectrometer. Obtained compound of ethyl acetate fraction number 21. With a value of CC to a WiDr cell of 11.24 ± 3.44 µg/mL and 342. 22 ± 4.98 µg/mL to Vero cells.

Keywords: *Zingiber purpureum Roxb., WiDr, Vero,*

Introduction

Colorectal cancer ranked fourth in all men and women worldwide, with an incidence of 1.400,000 new cases diagnosed in 2012 [1]. IARC states that cancers of the colon and rectum are the most common incidence of cancer with the number 3 (34,000 per year, 15.9%) and the third cause of death (18,000 per year, 10.8%). Chemotherapy was one of the therapies in the treatment of cancer. Chemotherapy agents generally have low selectivity properties because they are antiproliferative to normal selkanker and cell [2]. In addition, some chemotherapy has a narrow therapeutic index, and may lead to multidrug resistance (MDR) and adverse side effects [3].

One of the problems that often arise in the treatment of cancer is the resistance of chemotherapy drugs (drug resistance). Doxorubicin is a chemotherapy agent of various types of cancer of the anthracycline class that has provoked resistance. In addition to causing resistance, doxorubicin may also cause cardiotoxicity in long-term use [4]. Increased doses of doxorubicin are not an appropriate way to overcome resistance problems because they can cause normal cells to be exposed by cytotoxic drugs that will trigger toxicity and transform normal cells into cancer cells [5].

One of the medicinal plants in Indonesia that potentially developed as a chemopreventive agent in colon cancer is bangle. The bangle plant has been used by society traditionally to cope with several diseases. Empirically, boiled water rhizome bangle used the community as a headache medicine, constipation drugs, abdominal pain, jaundice, as body warmers, slimming, helps the gas out of the digestive tract, asthma, rheumatism, and antipyretics.

The research on scientific proof of the efficacy of the rhizome of bangle plant is still minimal publication and the efficacy of rhizome of bangle plant still many that have not been explored. So in this study, conducted an active fraction search on the ethanolic extract

of rhizome plant bangle that can kill and suppress the growth of cancer cells, especially in colon cancer cell type WiDr.

Material and Instrument

Methanol, Hexane and ethyl acetate fraction faloak bark, DMSO 0,1%, cisplatin (Wako),), (FBS) % (v/v) (Qualified , Gibco, Invitrogen USA), penicillin-streptomycin 1,5% (v/v) (Gibco, Invitrogen USA and Fungizone 0,5% v/v Gibco), Tripsin-E A 0,25% (Gibco, Invitrogen Canada),) MTT prepared with a concentration of dissolved) 1 x 0, 01 N HCl (Mer , Darmstadt, Germany), PBS containing).

Liquid nitrogen, cabinets ( ), micropipet (la balance sheets , Iowa, Benchmark), FACTS calibur Biospectrometry NMR 400MHz ^{1}H- ^{13}C-), mm2,), stirrer (Nuova, Thermolyne).

Procedure

Plant Material

(250 g) ethanol maceration method.

filtere

Fractionation with Column Chromatography

Separation of the active compound GF_{254} and the 200 mL n-hexane, ethyl acetate and methanol as motion phase was carried out gradiently with the addition of 1 drop of CH_3COOH. Each fraction obtained was evaporated at ± 40 °C to obtain a concentrated fraction and then dried in an oven at ± 50 °C. Each fraction tested to the cytotoxic activity to obtain the value of CC_{50}.

each fraction DMEM %.

Isolation and Purification under Bioassay-Guided Screening

According to cytotoxicity test UV, infrared, LC-MS one (ethyl acetate fraction 21). The fraction (4:7)

Determination of Cell Cycle and Apoptosis

[redacted]

Result and Discussion

Results of Cytotoxic Test

The cytotoxic activity of the fraction was expressed as CC_{50}. The test was performed by dissolving 14 fractions of fractionation of ethanolic extract with gradient system (n-hexane, ethyl acetate and methanol), then into it added DMSO solution until obtained the desired concentration. A single cytotoxic effect showed decreased cell viability and morphological changes in colon cancer cells of WiDr. The test results (Fig.1) show that Ethyl Acetate Faction 21 treatment (Fig. 1b-c) shows the wrinkled cell. This indicates a decrease in living cells when compared to controls (Fig. 1a).

[redacted] appear [redacted] to indicate [redacted] unknown changes were due to cell deaths due to necrotic or apoptotic processes and proliferative inhibition processes. Meanwhile, the CC_{50} value of the largest was ethyl acetate faction 21 with concentrations of 11.24 ± 3.44 µg/mL and doxorubicin was 13.88 ± 0.34 µg/mL.

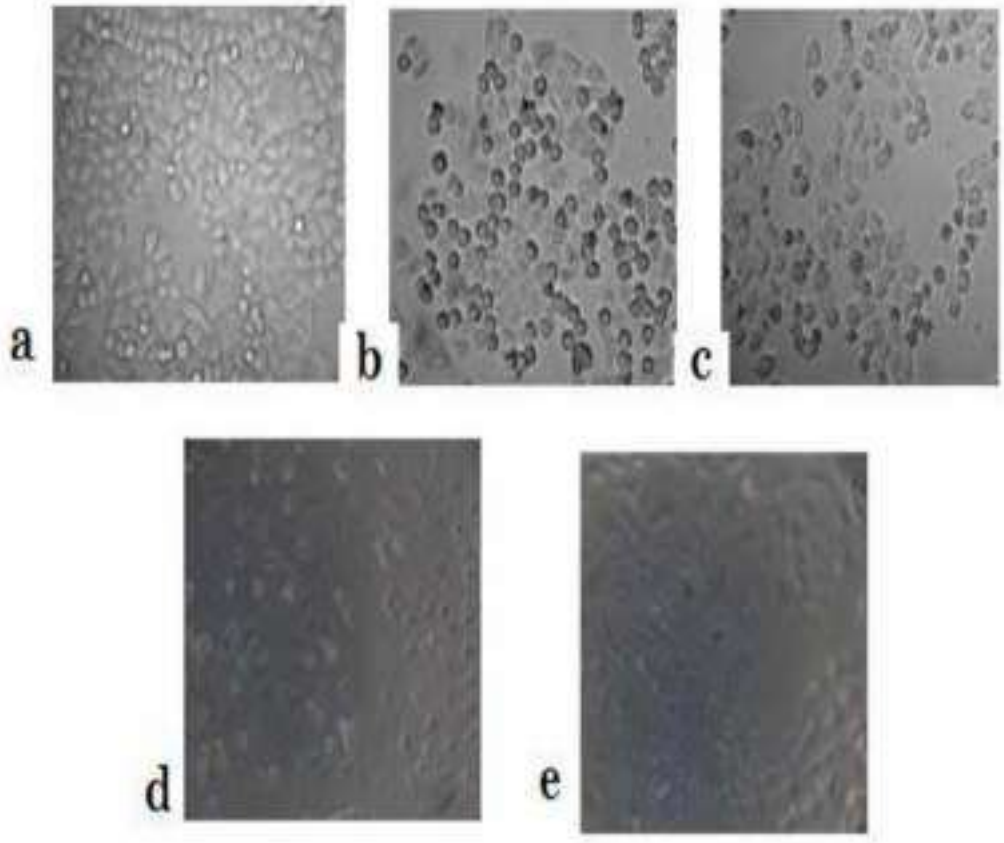


Fig.1: Ethyl Acetate Faction 21 testing was performed using cell controls (a) and Vero cells (d) Effect of Ethyl Acetate Faction 21 o[redacted]WiDr colon cancer cells (b-c). Figure (b) at concentrations of 200 µg/mL and image (c) [redacted] Vero cells [redacted] treated with a fraction of concentration of 200 µg/mL.

Table 1: Cytotoxic activity on WiDr and Vero

Number	Experiment	CC_{50} on WiDr Cell	CC_{50} on Vero Cell
1	Doxorubicin	13,88±0,34 µg/mL	160,67± 76 µg/mL
2	Methanol fraction 1	56,54 ±1,67	240,89 ± 96 µg/mL
3	Methanol fraction 2	70 92	140,92 ± 1,22 µg/mL
4	Methanol fraction 3	31,22 ±2	341,67 ± 2,39 µg/mL
5	Methanol fraction 4	48,88 ± 88 µg/mL	131,33 ± 1,96 µg/mL
6	Methanol fraction 5	74,38 ± 55 µg/mL	402,43 ± 1,07 µg/mL
7	Methanol fraction 6	99,68 ±0,87 µg/mL	148,56 ± 3,84 µg/mL
8	Methanol fraction 7	58,33 ±1,99 µg/mL	340,44 ± 1,45 µg/mL
9	Ethyl Acetate Faction 1	41,11 ±2,68 µg/mL	441,44 ± 0,34 µg/mL
10	Ethyl Acetate Faction 2	66,78 ±1,03 µg/mL	561,90 ± 1,52 µg/mL
11	Ethyl Acetate Faction 3	36,99 ±0,86 µg/mL	445,95 ± 1,14 µg/mL
12	Ethyl Acetate Faction 4	98,82 ±3,35 µg/mL	123,98 ± 3,22 µg/mL
13	Ethyl Acetate Faction	95,85 ±3,08 µg/mL	248,07 ± 2,58 µg/mL
14	Ethyl Acetate Faction 6	88,55 ±2,88 µg/mL	338,77 ± 4,95 µg/mL
15	Ethyl Acetate Faction 7	13,11 ±2,08 µg/mL	565,44 ± 1,08 µg/mL
16	Ethyl Acetate Faction 8	36,88 ±1,07 µg/mL	341,69 ± 0,11 µg/mL
17	Ethyl Acetate Faction 9	32,54 ±1,91 µg/mL	249,22 ± 0,65 µg/mL
18	Ethyl Acetate Faction 10	16,22 ±0,79 µg/mL	241,53 ± 0,96 µg/mL
19	Ethyl Acetate Faction 11	99,11 ±2 16 µg/mL	299,19 ± 4,98 µg/mL
20	Ethyl Acetate Faction 12	88,19 ±5,	349,93 ± 0,96 µg/mL
21	Ethyl Acetate Faction 13	14, ±4,33	240,11 ± 1,93 µg/mL
22	Ethyl Acetate Faction 14	88, ±3 16	360,24 ± 5,11 µg/mL
23	Ethyl Acetate Faction 15	16,92± 2,	130,11 ± 8,22 µg/mL
24	Ethyl Acetate Faction 16	28, ±3,30	223,11 ± 2,30 µg/mL
25	Ethyl Acetate Faction 17	92,22 ±3,93 µg/mL	392,12 ± 2 16 µg/mL
26	Ethyl Acetate Faction 18	31,21 ±3,33 µg/mL	234,44 ± 4,
27	Ethyl Acetate Faction 19	39,92 ±4,22 µg/mL	234 ± 3 16
28	Ethyl Acetate Faction 20	21,22 ±2,22 µg/mL	134,21 ± 3,
29	Ethyl Acetate Faction 21	11,24 ±3,44 µg/mL	342 ± 4,98
30	Ethyl Acetate Faction 22	41,98 ±9,92 µg/mL	232,22 ± 1,92 µg/mL
31	Ethyl Acetate Faction 23	34,22 ±2,33 µg/mL	234,03 ± 3,22 µg/mL
32	Ethyl Acetate Faction 24	92,23 ±2,22 µg/mL	445,11 ± 2,39 µg/mL
33	Ethyl A te Faction 25	82,11 ±3	354,73 ± 1,39 µg/mL
34	Hexane	77,92 ± 93 µg/mL	551,82 ± 3,11 µg/mL
35	Hexane	98,28 ±3,34 µg/mL	458,11 ± 1,28 µg/mL
36	Hexane	90,01 ± 88 µg/mL	388,29 ± 3,29 µg/mL
37	Hexane	34,22 ±1,	123,21 ± 2,92 µg/mL
38	5	22,11 ± 62 µg/mL	220,91 ± 0,16 µg/mL
39	6	97,14 ± 62 µg/mL	240,11 ±
40	7	36,22 ±4,11 µg/mL	233,11 ± 92 µg/mL
41	8	90,11 ± 66 µg/mL	102,11 ± 81 µg/mL
42	9	83,29 ± 98 µg/mL	358,09 ± 0,12 µg/mL
43	10	73,12 ± 93 µg/mL	241,72 ±
44	11	99,54 ±1,67 µg/mL	229,11 ± 88 /mL
45	12	96,11 ±2,22 µg/mL	221,11 ± 0,11 µg/mL

Structure Elucidation

Compound 1, yellow crystal, m.p. 97-99 °C, UV (MeOH) λ_{max}, nm (log ε): 232 (3.92), 290 (4.10), dan 325 sh (3.66) nm. EIMS: *m/z* (270, M^+, 100, base peak), 193 (76.1), 166 (80.3), 138 (41.4), 10^3 (21.2), and 77 (16.4). ^{1}H NMR (400 MHz in acetone *d6*), δ_H ppm: 12.17 (1H, *br, s*, 5-OH), 7.58 (3H, *m*, H-3',4',5'), 7.41 (2H, *m*, H-2',6'), 6.09 (1H, *d*, *J*=2.2 Hz, H-8), 6.07 (1H, *d*, *J*=2.2 Hz, H-6), 5.61 (1H, *dd*, *J*=3.8; 12.3 Hz, H-2), 3.35 (3H, *s*, 7-OCH_3), 3.24 (1H, *dd*, *J*=12.8; 16.4 Hz, H-3$_{ax}$), and 2.81 (1H, *dd*, *J*=3.8; 16.4 Hz, H-3$_{eq}$). ^{13}C NMR (100 MHzin acetone d6), δ_C ppm: 196.3 (C-4), 168.6 (C-7), 164.8 (C-5), 163.3 (C-8a), 140.1 (C-1'), 129.6 (C-3',5'), 128.5 (C-4'), 123.9 (C-2',6'), 101.5 (C-4a), 95.09 (C-8), 95.66 (C-6), 79.6 (C-2), 56.1 (7-OCH_3), and 43.9 (C-3). The elucidation results showed that the compound of the ethyl acetate fraction 21 was 2,3-dihydro-5-hydroxy-7-methoxy-2-phenylchromen-4-one.

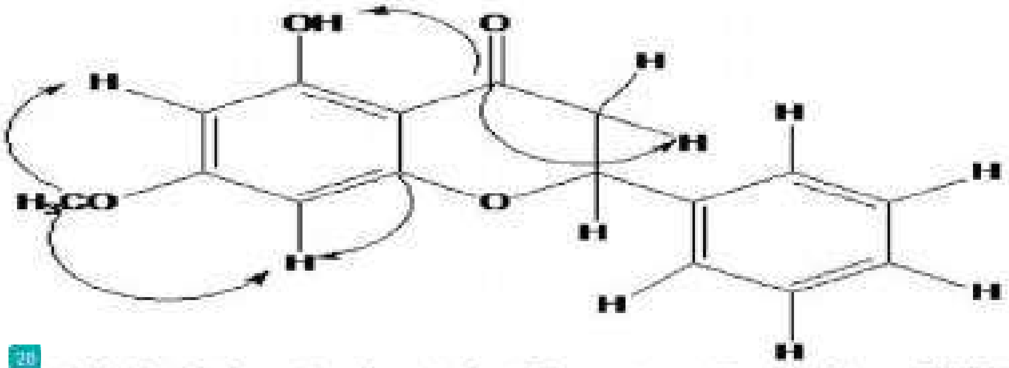


Fig. [redacted] Compound (HMBC)

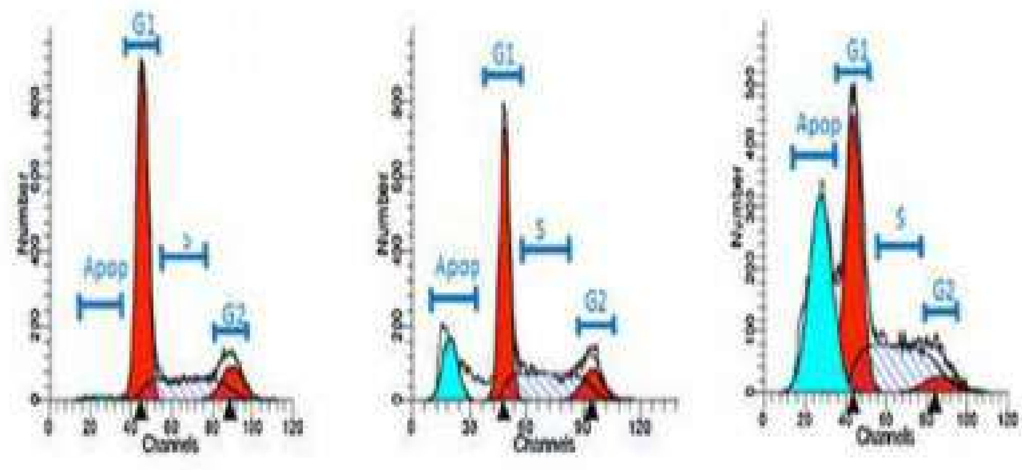
Observation of [redacted]

Furthermore, [redacted] test of cell cycle. The process of [redacted] cells [redacted] same cell [redacted] as normal cells [6]. [redacted] inhibiting [redacted] cell [redacted] is the modulation of cell cycle that can be observed using flowcytometry method (Rollando, 2000).

Flowcytometry is [redacted] each [redacted] cell [redacted] the number of chromo[redacted]omes on [redacted]) [7]. [redacted] is [redacted] becaus[redacted] is capable of interacting with DNA [8]. [redacted] profile [redacted] 24 hours [9]. [redacted] percentage of cell cycle distribution in detail is shown in Table 2.

Table 2: Percentage of cell cycle distribution

Sample	G1 (%)	S (%)	G2/M (%)	% CV
Control	38,11	12,34	13,34	3,87
Doxorubicin 2,5 µM	42,88	13,11	24,55	2,22
2,3-dihydro-5-hydroxy-7-methoxy-2-phenylchromen-4-one 2,5 µM	34,11	23,11	11,23	5,98



(a) (b) (c)

Fig. 3: Distribution of cell cycle percentages. (a) Control (b) Doxorubicin (c) [redacted] Compound

[redacted] test results showed doxor[redacted]icin causing accumulation in phase S. The [redacted] compound causes cell accumulation in phase [redacted] when compared with cell control. In the [redacted]

in amounted to 23.11% greater than the single doxorubicin of 13.11%. Cell accumulation in the S-phase of combination was increased compared with cells without treatment (cell control) of 12.34%. Cell accumulation is possible because of cell cycle arrest in that phase.

Table 3: Percentage of death after treatment

	Control	Doxorubicin 2.5 µM	2,3- 2.5 µM
Initial apoptosis (%)	2,11	2,33	9,87
End apoptosis (%)	1,63	2,53	1,24
Necrosis (%)	0,86	0,87	1,62
Total	3,94	6,04	13,78

Observation of apoptotic compounds flow cytometer. Induction apoptosis was used to investigate the mech mechanism of cell death from treatment of the compound on colon cancer cell WiDr incubated for 24 hours [10].

The study is flowcytometry see of apoptosis th occurred in the treated cells. Annexin V phospholipid binding are on strongly negatively charged cellular membranes. Cell cell death caused by apoptosis or necrosis can be distinguished by the staining of Propidium Iodide (PI) through intercalation with DNA [11]. T percentage of cell death after treatment of induced 3.

nalysis of the percentage of cell death after (Table 3) showed that control cells showed a 3.94% percentage of cell death. Single-treated cells with doxorubicin showed 6.04% cell death, whereas 2, 3-dihydro-5- hydroxy-7- methoxy-2-phenylchromen-4-one compound resulted in 13.78% cell death. It showed an increase in the percentage of cell death by 7.74% in the 2,3-dihydro- 5-hydroxy- 7-methoxy- 2-phenylchromen-4-one compound compared with doxorubicin.

Conclusion

The results showed that isolate 2 was 3-dihydro-5-hydroxy-7-methoxy-2-phenylchromen-4-one having activity cytotoxic in breast cancer cell type T47D with active category with IC_{50} 11, 24±3,44 µg/mL.

Acknowledgements

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7