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THE ACTIVITY OF EURYCOMANONE DERIVATIVES ON CANCER CELL LINES

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ABSTRACT: Eurycomanone from the roots of *Eurycoma longifolia* Jack (ELJ) has been reported to exhibit anticancer activity. Four of ester eurycomanone derivatives: eurycomanone dibutyrate, eurycomanone monovalerate, eurycomanone dimethoxybenzene, and eurycomanone disuccinate were synthesized for knowing their activities on cancer cell lines T47D, MCF-7, Hela, WIDR and Vero cells. The cancer activity was evaluated by the MTT colorimetric assay method. The results showed that eurycomanone has anticancer activity on T47D, MCF-7, Hela, WIDR cancer cells with IC_{50} values (1.17 ± 0.09; 3.96 ± 0.02; 2.95 ± 0.08; 1.45 ± 0.01 µg/mL), and no toxic to Vero cells (609.89 ± 29.77 µg/mL). Its derivatives eurycomanone dibutyrate have anticancer activity on T47D, MCF-7, Hela, WIDR cancer cells with IC_{50} values (25.16 ± 2.25; 21.56 ± 4.55; 29.32 ± 1.25; 149.42 ± 12.50µg/mL), eurycomanone monovalerate (25.59 ± 1.31; 22.48 ± 1.25; 30.14 ± 1.89; 91.88 ± 8.90 µg/mL), eurycomanone dimethoxybenzene (102.77 ± 2.56; 38.83 ± 2.55; 66.65 ± 1.90; 51.61 ± 2.37µg/mL), eurycomanone disuccinate (218.94 ± 9.30; 198.87 ± 5.50; 166.67 ± 12.34; 145.39 ± 6.67 µg/mL) respectively. The conclusion of this study showed eurycomanone dibutyrate, eurycomanone monovalerate have low activity as anticancer and safe to Vero cells. Eurycomanone dimethoxybenzoate are safe to Vero cells, besides that eurycomanone disuccinate toxic to Vero cells.

INTRODUCTION: Natural compounds from plants have been proven as a source of lead compounds for developing new drugs [1, 2]. Eurycomanone is a natural pentacyclic quassinoid obtained from the roots of *Eurycoma longifolia* Jack in the family of Simaroubaceae [3]. This popular plant uses as an aphrodisiac and the roots usually taken for preparing pharmaceutical products [4].

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Various natural compounds have been isolated and characterized, mostly from the extract of the ELJ roots. The compounds have been reported to have a wide pharmacological activities such as anti-malarial, anticancer and antipyretic and anti-ulcer [5]. Eurycomanone is one of the major natural quassinoids isolated from the roots and has exhibited cytotoxic activities against selected cancer cell line [3, 4].

Its pharmacological potency has been proven in numerous *in-vitro* and *in-vivo* experimental laboratory. But until now very limited efforts to develop this compound for obtaining its derivatives. Therefore, this study was conducted for knowing the anticancer activity of synthesized

eurycomanone derivatives on selected cancer cells lines (T47D, MCF-7, Hela, WIDR) and Vero cells by MTT Colorimetric Assay Methods [6]. The synthesis of eurycomanone derivatives was done by using eurycomanone natural compound and esterified without using a protecting agent. Esterifying of eurycomanone by using pharmacophore agents such as butirylchloride, valeroylchloride, para methoxy benzoyl chloride, and succinate anhydride. This study was approved by The Institutional Ethics Commission, Faculty of Medicine Universitas Syiah Kuala with number: 46/KE/FK/2017).

MATERIALS AND METHODS:

Materials: The plant and roots of ELJ were taken after 4 years of cultivation and identified by a specialist. The certificate of identification with number BF/123/Ident/Det/III/2010. The used of pharmacophore agent in this study (butirylchloride, valeroylchloride, para methoxy benzoyl chloride, and succinate anhydride) were pro analytic grade.

Methods: The roots of ELJ (10kg) were cleaned with tap water and then dried in the oven at 40 ºC. After cutting in small pieces, the dried roots were ground into a crude powder and stored in the desiccators. Then the crude powder was soaked in 30L methanol at room temperature and stirred regularly. The liquid extract was filtered and concentrated in a rotary evaporator at 40 ºC to produce the methanolic extract.

Isolation of Eurycomanone: Before isolation the methanolic extract of ElJ was subjected to vacum liquid chromatography (VLC) by using stationary phase silica gel and the mixed mobile phase chloroform: methanol: water in the ratio (5:5:1; 3:7:1: 1:9:1). Fractions with similar Rf values on thin layer chromatography (TLC) which were monitored at UV lamp at 254 nm, then pooled and used for isolation of eurycomanone as starting material for synthesizing its derivatives.

Isolation of eurycomanone was done by preparative thin layer chromatography (PTLC) using silica gel PF_{254} as stationary phase and the mixed mobile phase ethyl acetate: methanol: water in ratio 80: 20: 1. Repeated isolation, purification, and crystallization were done to get the pure compound. The structure and its purity were confirmed by comparison with detailed spectroscopic data in published reports (UV, IR, NMR, LCMS-ESI positive ion, DEPT, COSY, HMQC, and HMBC).

Synthesis of Eurycomanone Derivatives: Synthesis of eurycomanone derivatives can be performed by simple esterification without using a protecting agent. Eurycomanone which is isolated from the extract of ELJ roots (50 mg, 0,1225 mmol) was dissolved in cold pyridine (1 mL) and pharmacophore agent (butiryl chloride, valeroyl chloride, para methoxy benzoyl chloride, and succinate anhydride 0.49 mmol, respectively) dissolved in cold chloroform. The solution of pharmacophore agent was added slowly to the eurycomanone solution at 0 ºC, and the reaction mixture was stirred for 1 hour in an ice bath. After that, the reaction mixture was refluxed and stirred using magnetic heat stirrer for 6-8 h, and every 2 h checked the product by TLC.

After the esterification process ended, the mixture was extracted three times with 10 ml of cold ethyl acetate. The ethyl acetate layer is washed three times with 10 mL cold water and then dried with sodium sulfate anhydrate. After filtration and drying, the cooled precipitate is poured in methanol and preparing for detailed spectroscopic analysis and used for anticancer activity testing.

Testing of Anticancer Activity: The anticancer activity test of eurycomanone and its derivatives on Vero cells and cancer cell lines (T47D, MCF-7, Hela, WIDR) were carried out by MTT Colorimetric Assay Methods. The tested compounds were used at concentration 25; 12,5; 6,25; 3,125; 1,57625, 0,78125 µg/mL and prepared from the substock solutions by serial dilution of media to give a volume of 100 µL in each microtitre plate well. The concentration of tested compounds was prepared in triplicate. As standard drug used doxorubicine and 5- fluorouracil in the same concentration. Then each well was added with 100 µL of 10^4 mL of cells in complete growth media, respectively. As controls were used the cells and media that were placed into 96 well microplates then incubated for 24 h at 37 ºC, 5% CO_2 and 90% humidity.

After incubation, the media was removed and 100 µL of the new medium and 10 µL MTT was added. Then, it was incubated again for 4 h and next to the media was aspirated and 100 µL SDS 10% in

0.001N HCl added. The microplate was re-incubated for 24 h in room temperature and its absorbance was read at λ 405 nm (Vero cells) and 595 nm (cancer cell lines) by ELISA reader. The IC_{50} value on Vero cells and cancer cell lines were determined by probit regression analysis.

RESULTS AND DISCUSSION: Firstly, after maceration, the solid extract of ELJ roots is yielded ± 6%, then after fractionation yielded ± 2.5% of eurycomanone fraction. Isolation of eurycomanone from this fraction was performed by preparative thin layer chromatography (TLC) and yielded ± 0.04%.

Eurycomanone is the potential quassinoid anticancer was structurally esterified by using acyl chloride and carboxylic anhydride to influence their activity and cytotoxicity to cancer cell lines and normal cells. Synthesis of its derivatives by esterification is attempted to increase activity, decreasing toxicity, or improving other pharmacological profiles. In finding new anticancer with better activity than the previous compound, it was esterificated OH group in eurycomanone structure by butiryl chloride, valeryl chloride, para-methoxybenzoyl chloride, and succinic anhydride. The result of esterification yielded eurycomanone dibutyrate (60.35%), eurycomanone monovalerate (55.10%), eurycomanone dimethoxybenzoate (60.10%) and eurycomanone disuccinate (65.25%). The chemical structure of all tested compounds had been analyzed by spectroscopic analysis.

Eurycomanone as starting material has formula $C_{20}H_{24}O_9$ (MW 408.02; m.p 254º-257 ºC) and its derivatives eurycomanone dibutyrate $C_{28}H_{36}O_{11}$ (MW 548,94; m.p 241-243 ºC), eurycomanone monovalerate $C_{25}H_{32}O_{10}$ (MW 492,8; m.p 235-237 ºC), eurycomanone dimethoxybenzoate $C_{36}H_{36}O_{13}$ (MW 676.13; m.p 225-228 ºC) and eurycomanone disuccinate $C_{28}H_{30}O_{15}$ (MW 606,86, m.p 251-254 ºC). The evaluation of tested compounds on Vero cell is aimed for knowing the safety of these compounds on normal cells. In addition, these compounds are also used for examining their activities growth inhibition of cancer cell lines.

The IC_{50} and selectivity index values of these compounds on cancer cell lines and Vero cells are showed in **Table 1** and **2**.

TABLE 1: THE IC_{50} OF EURYCOMANONE AND ITS DERIVATIVES ON SELECTED CANCER CELL LINES AND VERO CELLS

Tested compounds	IC_{50} Values (µg/mL)				
	Vero	T47D	MCF-7	Hela	WIDR
Eurycomanone	609.8 ± 29.7	1.1 ± 0.1	3.9 ± 0.0	2.9 ± 0.0	1.4 ± 0.0
E. dibutyrate	219.2 ± 11.3	25.1 ± 2.2	21.5 ± 4.5	29.3 ± 1.2	149.4 ± 12.5
E. monovalerate	92.4 ± 7.5	25.5 ± 1.3	22.4 ± 1.2	30.1 ± 1.8	91.8 ± 8.9
E dimethoxy benzoate	13.2 ± 6.9	102.7 ± 2.5	38.8 ± 2.5	66.6 ± 1.9	51.6 ± 2.3
E. disuccinate	12.7 ± 2.8	218.9 ± 9.3	198.8 ± 5.5	166.6 ± 12.3	145.3 ± 6.6
Doxorubicine	3.5 ± 0.6	1.9 ± 0.1	4.6 ± 0.1	3.5 ± 0.0	41.8 ± 2.2

TABLE 2: THE SELECTIVITY INDEX OF EURYCOMANONE AND ITS DERIVETIVES ON SELECTED CANCER CELL LINES

Tested compounds	Selectivity Indexes			
	T47D	MCF-7	Hela	WIDR
Eurycomanone	521.2	154.0	206.5	420.2
E. dibutyrate	8.7	10.1	7.4	1.4
E. monovalerate	3.6	3.4	3.0	1.0
E dimethoxy benzoate	128.7	340.6	198.4	256.2
E. disuccinate	0.0	0.0	0.0	0.0
Doxorubicine	1.8	0.7	1.0	0.0
5-Fluouracil	69.6	88.7	140.9	52.8

In-vitro screening of anticancer activity of eurycomanone and its derivatives is based on the ability of the compounds to inhibit the growth of cancer cell lines in medium culture. Previous studies showed the anticancer activity of eurycomanone on various cancer cell lines has IC_{50} value on MCF-7 is 4.40 ± 0.42 µg/mL [7]; 3.63 ± 0.11 µg/mL [8]; and less than 2.5 µg/mL [9]; 1.1 µg/mL [10]. The anticancer activity of eurycomanone on Hela cells has IC_{50} value 2.13 ± 0.09 µg/mL [7].

The result of the test toward four synthesized compounds showed that eurycomanone more potent than monoacetylated and diacetylated eurycomanone to selected cancer cell lines above. Some structural requirements, like an α, β-unsaturated ketone in the A ring, an oxymethylene bridge in the C ring and an ester function in C-15 in the D ring are considered very important for the anticancer activity which is presented by quassinoids [11, 12].

CONCLUSION: The data suggest that eurycomanone has anticancer activity more potential than its derivatives on selected cancer cell lines (T47D, MCF-7, Hela and WIDR) and safe to normal Vero cells. Eurycomanone dibutyrate, eurycomanone monovalerate and eurycomanone dimethoxybenzoate have a low activity as anticancer and safe to Vero cells with selectivity index (IS) more than 3, besides that eurycomanone disuccinate toxic to Vero cells.

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CONFLICT OF INTEREST: There is no conflict of interest in this study

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