Antiaging Potency of *Centella Asiatica* Extract on Fibroblast Cells of *Rattus Norvegicus* Fetus by in Vitro and in Silico Approach

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Abstract. Aging is a physiological process that cannot be avoided, but can be prevented by giving antioxidants. Antioxidants can be obtained from *Centella asiatica* extract (*EkCa*). *EkCa* has secondary metabolites that have biological effects related to inflammation and prevent aging. The purpose of this study was to determine the anti-aging potential of *EkCa* in fibroblast cells of *Rattus norvegicus* fetus and which compounds that act as anti-aging on *EkCa* using in vitro and in silico approach. The method used in this study was completely randomized design with 5 treatments (control, 10%, 15%, 20%, 25%), each repeated 4 times on fibroblast cells of *Rattus norvegicus* fetus that were 18 days pregnant. In vitro approach used to determine the confluency and viability on fibroblast cells of *Rattus norvegicus* while in silico approach used to screening which active compounds that have the potential as anti-aging using Way2Drug PASS online software. Data analysis used One Way ANOVA followed by Duncan's test. The results showed that the highest level of confluency and viability was found in the 25% treatment ($P < 0.05$) and quercetin-3- arabinoside was the compound in *EkCa* that has the potential as anti-aging with Pa: 0.922 and Pi: 0.003. From the result, it can be conclude that *EkCa* has the potential as anti-aging on fibroblast cells of *Rattus norvegicus.*

Keywords: Extract Centella asiatica (EkCa) · Rat Fetal Fibroblast Cells · Confluency · Viability · Screening

1 Introduction

Aging is a physiological process that cannot be avoided, but can be prevented by giving antioxidants. The occurrence of aging is triggered by free radicals in the body in the form of unpaired atoms or molecules [1]. Antioxidants can be obtained from *Centella asiatica* extract (*EkCa*) [2]. Several studies have shown that *EkCa* contains high

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antioxidants that are able to protect body cells from damage [3]. Muchtaromah (2019) said that compounds that have potential as antioxidants in *EkCa* are triterpenoid groups: asiatic acid, brahmoside, asiaticoside, schefforoside. Vitamins: chlorogenic acid, caffeic acid. Flavonoids: luteolin, kaempferol, kaemferol-3–0-rhamnoside, quercetin-3–0-rhamnoside. Saponins: centellasaponins [4]. Prestiyanti (2021) said that giving *Centella asiatica* paste 10% applied to the wound can increase the number of fibroblast cells by the in vivo method [5]. In contrast to previous studies, this study was used in vitro method using fibroblast cell culture media of *Rattus norvegicus* fetus with 5 treatments, namely: control, P1 = 10%, P2 = 15%, P3 = 20%, P4 = 25%. Each treatment repeated 4 times, while for screening of active compounds on *EkCa* that have the potential as anti-aging using Way2Drug PASS online software [6].

The purpose of this study was to determine the anti-aging potential of *EkCa* in fibroblast cells of *Rattus norvegicus* fetus and which compounds that act as anti-aging on *EkCa* using in vitro and in silico approach.

2 Methods

2.1 Extraction of Centella Asiatica, Preparation of DMEM Stock Media and Rat Fetus of Fibroblast Cell Culture

Extraction was made by soaking 100 g of *Centella asiatica* simplicia with 500 ml 70% ethanol (1:5) for 2 x 24 h, then filtered with filter paper and then evaporated using a rotary evaporator. Weighed 1.35 g DMEM, 0.37 g NaHCO3, 0.006 g penicillin, 0.01 g streptomycin and 0.23 g hepes. All these materials were dissolved with 100 ml of sterile deionized water (DI), then homogenized using a magnetic stirrer and filtered with a 0.22 μm millipore membrane. Then put in a screw cap bottle and stored at 4 °C [7].

The rat fetus at 18 days of gestation was isolated by cutting at the head and removing the internal organs. The fetal body was washed with 1% penstrep antibiotic (penicillin & streptomycin) in sterile PBS (0.5 ml penstrep in 50 ml sterile PBS). Organs were chopped in 500 μl trypsin until smooth, homogenized with a syringe, then put in a centrifuge tube. The remaining homogenization was added with 500 μl of PBS (1:1) then incubated for 20 min. The centrifuge tube was taken from the incubator and centrifuged at 2500 rpm for 5 min then the supernatant was removed and the pellet was added with 3 ml of DMEM media. Three ml of fungizone and 1 ml of penicillin streptomicin was centrifuged again at 2500 rpm for 5 min. The supernatant and pellet added 3 ml DMEM 10% FBS and 3 ml fungizone then centrifuged again. After that, the supernatant was discarded and 1 ml of the pellet was left and then pipetted. The resulting pellet was taken 50 μl and then put into multiwell 24 which already contained DMEM 10% FBS and fungizone. Then the cells were cultured at 37 °C, 5% CO^2 [8]. The treatment was carried out after the cell culture was confluent. This research has received the certificate of research ethics number: 029/EC/KEP-FST/2022.

2.2 Research Sample Division

This study was divided into 5 treatments and 4 replications with 48 h incubation. The each treatment as follows:

1. Group K: Fibroblast cells in DMEM media (2700 µl) + 10% FBS (300 µl) + 10% DMSO.
2. Group P1: Fbroblast cells in DMEM (2370 µl) + 10% FBS (300 µl) media with 10% *EkCa.*
3. Group P 2: Fibroblast cells in DMEM (2280 µl) + 10% FBS (300 µl) media with 15% *EkCa*
4. Group P 3: Fibroblasts in DMEM media (2190 µl) + 10% FBS (300 µl) with 20% *EkCa*
5. Group P 4: Fibroblast cells in DMEM (2100 µl) + 10% FBS (300 µl) media with 25% *EkCa*

2.3 Observation of Confluency and Viability of Fibroblast Cells with in Vitro Approach

Observation of confluency based on the level of cell attachment to the substrate and cell expansion using an Inverted Microscope and analyzed using the ImageJ program [9]. Fibroblast cell viability was calculated to determine the percentage ratio between living and dead cells. Cell viability calculation was carried out using counter cell. The procedure for observing cell viability was adapted to the Countess ™ II FL Automated Cell Counter User Guide (2019). A total of 10 µl of 0.4% trypan blue was added to 10 µl of cell suspension, then mixed well. Take 10 µl of the sample mixture and put it into the space on the slide, the mixture is allowed to stand for 30 s. The sample mixture is then inserted on the port slide on the instrument. The instrument will read automatically, adjust the focus and light intensity, then will show the number of cell concentrations, the percentage of cells that are alive and dead [10].

2.4 Screening of Active Compounds Contained in Centella Asiatica Extract (EkCa) with in Silico Approach

Screening of active compounds was carried out to determine which compounds that have the potential as anti-aging contained in *EkCa* with in silico approch using Way2Drug PASS Online software with ram 8 specifications [11].

2.5 Data Analysis

The data analysis of confluency and viability data were analyzed using One Way Anova followed by Duncan test using SPSS software. Meanwhile, in silico data was carried out by comparing the biological activity values from the screening of active compounds contained in *EkCa* [12].

3 Results and Discussion

3.1 Effect of Centella Asiatica Extract (EkCa) on Confluency and Viability of Fibroblast Cells

From the results of the study, the average yield on confluency and viability of fibroblast cells were shown in Fig. 1.

The results of research on the anti-aging potential of *EkCa* on fibroblast cells showed that there was a different effect on each treatment. The control showed the lowest confluency compared to P1(10%), P2(15%), P3(20%) and P4(25%) while P4 (25%) indicated the highest confluency value with an average of 265. This was evidenced by the analysis of One way ANOVA data.

From the ANOVA data, it showed that the $_{\text{calculated}}$ F F $_{\text{table}}$ with a significance of 5% ($\alpha = 0.05$), it was concluded that from 5 treatments and 4 replications each showed an effect on fibroblast cells confluency. Duncan's further test can be carried out to determine the effect of *Centella asiatica* (*EkCa*) extract on fibroblast cell confluency which shown in the Table 1.

The Table 1. Showed that giving 5 treatments and 4 replications each had an effect on the viability of fibroblast cells *Rattus norvegicus*. The control treatment showed the lowest cell viability compared to the treatment P1(10%), P2(15%), P3(20%) and

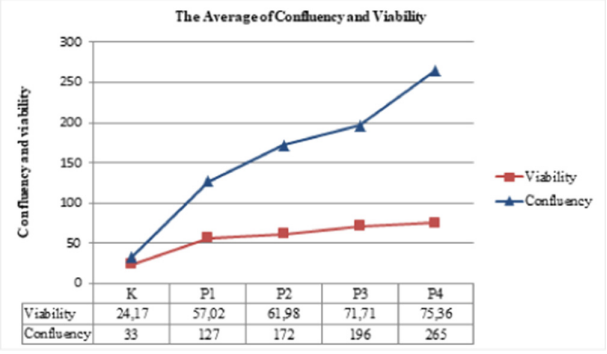

Fig. 1. Average confluency and viability of fibroblast cells *Rattus norvegicus*

Table 1. Summary of duncan 5% effect of *EkCa* on the confluency and viability of fibroblast cells

Treatment	Average confluency	Average Viability
K	33a	24,17a
P1 (10%)	127b	57.02b
P2 (15%)	172c	61.98c
P3 (20%)	196d	71.71d
P4 (25%)	265e	75.36e

Table 2. The results of screening active compounds that have the potential as anti-aging using Way2Drug PASS online software

No	Name	CID	Activity	Pa	Pi
1	p-coumaric acid A	637542	Response to UV B	0.641	0.024
2	p-coumaric acid	637542	Biosynthetic flavanoids	0.641	0.024
3	Luteolin	5280445	Flavone and flavanol biosynthesis	0.775	0.004
4	Kaempferol	5280863	Flavonoid biosynthesis process	0.856	0.003
5	Quercetin-3- arabinoside	12309865	Antioxidant	0.922	0.003
6	Quercetin-3-O rhamnoside	5353915	Antioxidant	0.915	0.003
7	Asiatic acid	119034	Antiinflammatory	0.399	0.012
8	Centellasapogenol A	73196815	Antiinflammatory	0.873	0.005
9	Methyl asiatate	21672634	Antiinflammatory	0.901	0.004

P4(25%). Treatment P4(25%) showed the highest cell viability with an average value of 75.36.

The ANOVA data showed F_{count} F_{table} with a significance value of 5% ($\alpha = 0.05$), it was concluded that giving 5 treatments and 4 replications each affected the viability of rat fetal fibroblast cells. To determine the effect of *EkCa* on the viability of fibroblast cells, Duncan's further test can be carried out, this was shown in the table 1.

3.2 Screening Results of Compounds that Have the Potential as Anti-Aging with Passonline Software

The results of the screening compounds that have the potential as anti-aging with Way2Drug PASS online software was shown in Table 2.

Screening results showed that there were 9 compounds that have the potential as antiaging. From the nine compounds, quercetin-3-arabinoside has the highest antiaging activity, with PA value: 0.922, Pi value: 0.003 while the asiatic acid compounds was the lowest with PA values: 0.399 and Pi: 0.012.

4 Conclusion

Based on the results of in vitro and in silico, it was concluded that the administration of *Centella asiatica* extract (*EkCa*) with 5 different treatments, each repeated 4 times showed antiaging potential, with confluency value: 265 and viability value: 75.36 at P4 treatment (25%). The results of the screening using the Way2Drug PASS Online software showed that quercetin-3-arabinoside had the highest antiaging activity, with Pa value: 0.922, Pi value: 0.003.

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