

# Efektivitas ekstrak centella asiatica dalam nanopartikel kitosan terhadap aktivitas proliferasi sel fibroblas dan keratinosit sintesis kolagen I, III dan ekspresi protein aquaporin-3 secara in vitro = The Effectiveness of centella asiatica in chitosan nanoparticles to the activity of cell proliferation collagen synthesis I,III and protein expression of aquaporin-3 in vitro

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## Abstrak

**Pendahuluan:** Aktivitas proliferasi, sintesis kolagen, dan hidrasi kulit akan menurun seiring proses penuaan kulit, sehingga kulit menua menjadi kusam, kendur, dan kering. Aquaporin-3 (AQP3) adalah protein kunci yang berperan pada proliferasi dan hidrasi keratinosit, sekarang menjadi target inovasi pengembangan kosmetika pelembab anti penuaan kulit. Penelitian ini bertujuan menganalisis kombinasi dua bahan alam yaitu ekstrak etanol *Centella asiatica* dan nanopartikel kitosan (EECA+NPK) terhadap proliferasi sel fibroblast dan keratinosit, sintesis kolagen I dan III serta ekspresi protein aquaporin-3 (AQP3) secara in vitro.

**Metode:** Dilakukan uji proliferasi sel fibroblas dan keratinosit yang dianalisis dengan uji Microculture Tetrazolium (MTT), analisis sintesis kolagen I dan III menggunakan Enzyme Linked Immunosorbent Assay (ELISA) kit (COL1A1 dan COL3A1) setelah dipajankan dengan ekstrak etanol *Centella asiatica* dalam nanopartikel kitosan (EECA + NPK) pada beberapa konsentrasi selama 24, 48, dan 72 jam dibandingkan dengan asam retinoat, selanjutnya ekspresi aquaporin-3 (AQP3) dianalisis dengan teknik Imunositokimia menggunakan antibodi anti-aquaporin3 ab125219, kemudian dianalisis secara kuantitatif menggunakan ImageJ software.

**Hasil:** EECA + NPK dapat meningkatkan proliferasi fibroblas 1.6 kali lipat dibandingkan kontrol. Optimal pada konsentrasi 6.25 mg/mL dan proliferasi sel keratinosit optimal pada konsentrasi 3.125 mg/mL, secara statistic tidak berbeda bermakna dengan AR. (EECA + NPK) dapat meningkatkan sintesis kolagen I setelah pajanan 72 jam dan kolagen III setelah pajanan 48 jam, secara statistic tidak berbeda bermakna dengan AR. Uji imunositokimia dengan antibodianti-aquaporin3 ab125219 (EECA + NPK) dapat meningkatkan ekspresi aquaporin 3 (AQP 3) pada sel fibroblas optimal pada konsentrasi 12.5 mg/mL dan sel keratinosit pada konsentrasi 3.125 mg/mL setelah pajanan selama 24 jam, secara statistik berbeda dengan AR.

**Kesimpulan:** Ekstrak etanol *Centella asiatica* dalam nanopartikel kitosan (EECA + NPK) dapat meningkatkan proliferasi fibroblas dan keratinosit, meningkatkan sintesis kolagen I dan III, serta ekspresi protein AQP3 pada sel fibroblas dan sel keratinosit.

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**Introduction:** Proliferation activity, collagen synthesis, and hydration of the skin will decrease with the process of aging, therefore, skin looks dull, sagging, and dry. Aquaporin-3 (AQP3) is a key protein that plays a role in keratinocyte proliferation and hydration, recently becomes the target of innovation

development of anti-aging cosmetic moisturizer. This research aims to analyze a combination of two natural ingredients, Centella asiatica ethanolic extract and chitosan nanoparticles (EECA + NPK) to increase proliferation of fibroblast cells and keratinocytes, synthesis of type I and III collagen, and the expression of AQP3 *in vitro*.

**Methods:** Microculture Tetrazolium Test was conducted to analyze the proliferation of fibroblasts and keratinocytes. The synthesis of type I and III collagen in fibroblasts was analyzed using Enzyme Linked Immunosorbent Assay (ELISA) kit (COL1A1 and COL3A1) after the exposure of CAEE + CNP in several concentrations for 24, 48, 72 hours and compared to Retinoic acid (RA). The expression of AQP 3 on fibroblasts and keratinocytes was analyzed using antibody anti-aquaporin 3 ab125219 immunocytochemistry technique, then quantitatively analyzed using Image-J software.

**Results:** CAEE+ CNP increased the proliferation of fibroblasts, optimal result at 6.25mg/mL concentration and the proliferation of keratinocytes increased 1.5 times than control, optimal result at 3.125 mg/mL concentration, statistically were not significantly different with RA. CAEE + CNP increased the synthesis of collagen type I, optimal after incubated for 72 hours and the synthesis of collagen type III, optimal 48 hours, statistically were not significantly different with RA. Using antibody anti-aquaporin 3 ab125219 immunocytochemistry examination indicated CAEE+ CNP increased the expression of AQP 3 on fibroblasts and keratinocytes, optimal results at 12.5 mg/mL and 3.125 mg/mL respectively after 24 hours exposure.

**Conclusion:** CAEE + CNP increased the proliferation of fibroblasts and keratinocytes, synthesis of type I and III collagen, and the expression of AQP3 in fibroblasts and keratinocytes; **Introduction:** Proliferation activity, collagen synthesis, and hydration of the skin will decrease with the process of aging, therefore, skin looks dull, sagging, and dry. Aquaporin-3 (AQP3) is a key protein that plays a role in keratinocyte proliferation and hydration, recently becomes the target of innovation development of anti-aging cosmetic moisturizer. This research aims to analyze a combination of two natural ingredients, Centella asiatica ethanolic extract and chitosan nanoparticles (EECA + NPK) to increase proliferation of fibroblast cells and keratinocytes, synthesis of type I and III collagen, and the expression of AQP3 *in vitro*.