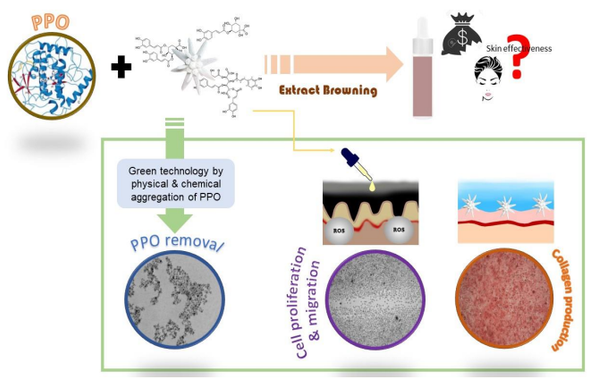
Green technology for browning prevention of Edelweiss extract in cosmetic formulation

NT_654

Suciati, T[1]*; Iftinan, F[1]; Putri, RA[1]; Nareswari, TL[1]; Juniatik, M[1]; Fidrianny, I[1]; Pamudji, JS[1]; Mauluddin, R[1]; Adlia, A[1]; Utami, D[2]; Rahmawati, Y[2]
[1]School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia; [2]Paragon Innovation Technology, Jakarta, Indonesia

Introduction:

The emerging use of edelweiss (Leontopodium alpinum Cass.) extract in beauty products is due to its pronounced antioxidant, anti-inflammatory, and anti-aging effects [1]. The aqueous extract contains various phenolic compound such as chlorogenic acid, 3,5-dicaffeylquinic acid, and leontopodic acid [2], which are susceptible to oxidation reactions catalyzed by polyphenol oxidase (PPO). The PPO reaction starts from a slow step to produce colorless ortho-diphenol, continues with a fast step to produce brown ortho-quinone, and ends with a protein conjugation reaction as a dark brown insoluble polymer.



Objective:

This research aimed to develop PPO removal following its precipitation & aggregation by:

i. chemical induction for hydrophobic amino acid exposure,
ii. physical aggregation using ultrasonication and rapid temperature change to increase the aggregation process.

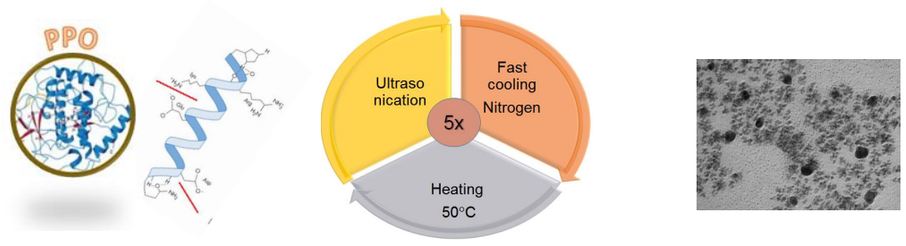
Materials & Methods:

Materials

Edelweiss extract (ALPAFLOR® EDELWEISS EP ex DSM Nutritional Products, LLC).

Methods

RCTU Treatment



40% extract in 2% citric acid → RCTU treatment → Removal of PPO precipitate

Browning prevention was conducted by PPO removal from edelweiss extract by:

i. Citric acid dilution of the extract to expose the hydrophobic amino acid by disrupting the electrostatic side chain and chelating activity to bind to the Cu (II) active side of PPO [3].
ii. Rapid temperature change with nitrogen immersion and heating at 30-50 °C to induce protein denaturation due to exposure to hydrophobic amino acids at the air-water interface [4,5].
iii. Ultrasonication to include energy input to intensify the aggregation process [6].

Characterization of the RCTU treated extract

The cycles of RCTU were determined from the highest amount of protein aggregate retained on the filter membrane and the lowest absorbance measured at 420 nm.

EXTRACT SUPERNATANT	PROTEIN PRECIPITATE
• Bradford protein assay by measurement of absorbance at 590 nm. • The total phenolic content was determined using Folin-Ciocalteu reagent and a standard gallic acid calibration curve, which was expressed as gallic acid equivalent per 100 g of extract.	• Morphology of the precipitate was revealed using transmission electron microscope (TEM Hitachi HT7700 • Bradford protein assay by measurement of absorbance at 590 nm.

Formulation and browning index analysis

Formulation: Untreated and treated extracts (4% v/v). Incorporated into the formulation chassis of face essence. Added antioxidants (ascorbic acid, tocopherol, and Na2EDTA) and adjusted to pH 6.

Stability testing: Stored at 40°C chamber for 2 months. The browning levels were analyzed by measuring the absorbance at 420 nm.

Biological activity of RCTU treated extract

Viability testing

- Fibroblast 3T3 cell lines were treated with untreated and the RCTU treated extracts at 3.125 to 100 µg/ml for overnight and analyzed using PrestoBlue™ viability assay.

Scratch test

- The cells were scratched, incubated in DMEM supplemented with 50 µg/ml extract for 2 days and stained using picrosirius red for collagen expression analyses. The healing areas were analyzed using ImageJ.

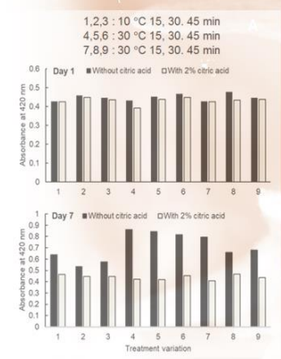
Results & Discussion:

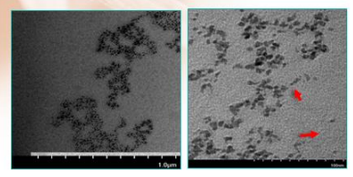
PPO AGGREGATION & REMOVAL

Chemical treatment using 2% citric acid suppressed the browning level during heating & ultrasonitaion treatment.

Furthermore, 5 cycles of physical treatment by ultrasonication for 15 min with rapid temperature changes by quenching at -10 to -15 °C using nitrogen immersion for 1 min followed by heating at 50 °C maximized PPO aggregation & precipitation.

The large aggregates are mostly found, but some small aggregates are formed and potentially pass to the filtrate which contaminates the final cosmetic product (red arrow).



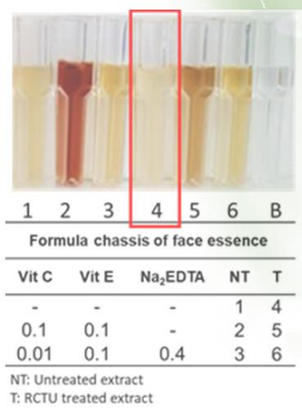


TEM images of PPO aggregates produced after RCTU treatment of 2% citric acid diluted edelweiss extract.

FORMULATION & BROWNING INDEX

To anticipate the PPO residual in the RCTU processed extract, various antioxidants were added in the formula chassis of face essence. The addition of antioxidants adversely increased the browning reaction.

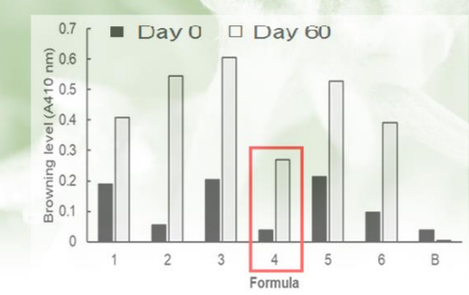
Importantly, the formula of treated extract without antioxidant supplementation had the lowest absorbance and clearest appearance.



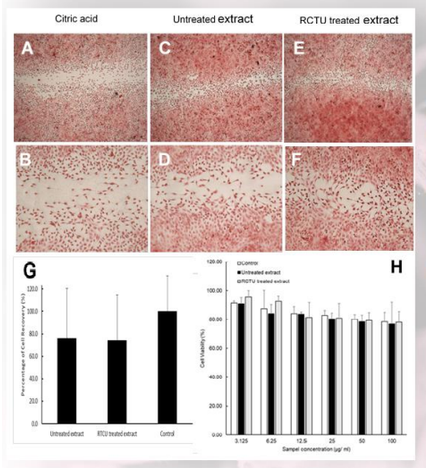
Formula chassis of face essence

Vit C	Vit E	Na_2EDTA	NT	T
-	-	-	1	4
0.1	0.1	-	2	5
0.01	0.1	0.4	3	6

NT: Untreated extract
T: RCTU treated extract



PHENOLIC CONTENT & BIOLOGICAL ACTIVITY



Phenolic content and biological activity to fibroblast cell culture in vitro were maintained after the RCTU treatment of edelweiss extract :

(i) The proliferation and migration for cell recovery two days after 48 hours of scratch test visualized by picro-sirrius red staining (A to G) and

(ii) The cell viability 24 hours after treatment of citric acid (control), untreated and RCTU treated extract.

Total phenolic content of edelweiss extract

Sample	Phenolic content (µg/ml)
Without treatment (control)	1238.57 ± 126.32
With RCTU treatment	1177.28 ± 76.88

Conclusions:

The browning reaction due to polyphenol oxidase (PPO) activity in edelweiss cosmetic products was prevented by several strategies:

- Liquid nitrogen induced PPO denaturation in 40% aqueous extract acidified with 2% citric acid.
- A series of rapid temperature changes and ultrasonication (RCTU process) at 5 cycles resulted in PPO aggregation and precipitation.
- PPO aggregates was discharged from the extract using membrane filtration.
- Polyphenol content of the extract remained stable after the processing that maintaining cellular growth and collagen stimulation assessed on fibroblast cell culture in vitro.

Acknowledgments:

Parts of this research were funded by Paragon Innovation Technology and the cell culture works were funded by School of Pharmacy.

References:

1. Dobner MJ, Sosa S, Schwaiger S, Altinier G, Della Loggia R, Kaneider NC, Stuppner H (2004) Anti-inflammatory activity of Leontopodium alpinum and its constituents. Planta Med 70(06):502-508.
2. Schwaiger S, Cervellati R, Seger C, Ellmerer EP, About N, Renimel I, Godenir C, Andre P, Gafner F, Stuppner H (2005) Leontopodic acid—a novel highly substituted glucaric acid derivative from Edelweiss (Leontopodium alpinum Cass.) and its antioxidative and DNA protecting properties. Tetrahedron, 61(19): 4621-4630.
3. Moon KM, Kwon EB, Lee B, Kim CY (2020) Recent trends in controlling the enzymatic browning of fruit and vegetable products. Molecules *25*(12):2754.
4. Rosa M, Roberts CJ, Rodrigues MA (2017) Connecting high-temperature and low-temperature protein stability and aggregation. PloS one, 12(5):e0176748.
5. Martin-Diana AB, Rico D, Barry-Ryan C, Mulcahy J, Frias J, Henehan GT (2005) Effect of heat shock on browning-related enzymes in minimally processed iceberg lettuce and crude extracts. Biosci Biotechnol Biochem 69(9):1677-1685.
6. Gani A, Baba WN, Ahmad M, Shah U, Khan AA, Wani IA, Masoodi FA, Gani A (2016) Effect of ultrasound treatment on physico-chemical, nutraceutical and microbial quality of strawberry. LWT-Food Sci Technol 66:496-502.