# Development of a new sustainable cosmetic ingredient using wild rose callus originated from Yunnan Shangri-la

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## Introduction:

Plant cell culture technology (PCT), which is a highly efficient process with advantage of less energy cost, lower impacts on the environment, and independent of location and season, has been more and more widely utilized as an natural and sustainable approach in medical and cosmetic manufacturing. The genus *Rosa* (roses) is well known as an ornamental plant, which also has been used in folk medicine or cosmetic industry worldwide for centries. *Rosa graciliflora* (Rg) is a wild rose growing in picea forests or scrub at forest margins at altitudes ranging from 3300 to 4500 m, and it is particularly distributed in the parts of Yunnan, Tibet and Sichuan of China. However, there has been few reports on the research of this wild rose specie. Therefore, the objective of this study was to develop a sustainable ingredient from Rg callus orginated from Yunana Shanggri-la by PCT, and meanwhile to explore its skin care efficacy to provide fundamental support for its application in cosmetics.

## Materials & Methods:

**1.Production of *Rosa graciliflora* callus**

*Rosa graciliflora* fruit pods (Fig.1A) were collected in Shangri-la, Yunnan, China. The seeds firstly were carried on the China's satellite,Shijian-10 in 2016. Secondly, the seeds were stored in sand with at 5 ℃ for 9 months. After seeds germinating (Fig.1B), the *Rosa graciliflora* stems were cut and sterilized,cultured on basic Murashige and Skoog (MS) medium containing 6-Benzylaminopurine (6-BA) and α-Naphthalene acetic acid (α-NAA). After 3 weeks young leaves of asepyic seedling (Fig.1C) were cut from stem for callus induction.The Rg callus was and proliferated on MS medium containing deionized water or glacier water. Glacier water is used to proliferate more Rg callus. All plants or callus were grown under weak light (irradiance of 20 μmol $m^{-2}$ $s^{-1)}$ at (20±1) ° C with a 12-h photoperiod. Finally, the Rg callus was harvested and grinded in distilled water, and following ultrasonic extraction for 30 min. After centrifuge and filtration, the filtrate was dired by freeze drier and the Rg callus extract (RCE) powder was obtained. The powder was dissolved in phosphatic buffer solution (PBS) at concentration of 1000 μg/mL for the further assessment.

**2.DPPH radical scavenging experiment**

Anti-oxidant effects of RCE were examined by the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay. The absorbance was measured by spectrophotometer at 525nm and the activity was calculated as below:

DPPH Scavenging activity (%) =[1-(T-T0)/(C-C0) ]*100

**3.2D cell assays**

Human keratinocyte (HaCaT) cells and human fibroblasts (FB) were seeded in the plate for 24 h with MEM and DMEM plus 10% fetal calf serum (FCS) respectively. And the cells were treated at final different concentrations of RCE for 24 h. Same volume of PBS was used as a vechile control. Similarly, Hydrogen peroxide ($H_2O_2$) induced cellular oxidative damage assay, UVB irradiation damage recovery and Micronuleus assay, Enzyme-linked immuno sorbent assay (ELISA) for Human type I collagen synthesis, ATP synthesis, ELISA for UVB-induced inflammatory cytokines were detected by using HaCat cells and FB cells.

**4.3D skin model**

The senescent keratinocytes were inoculated into 12 well Transwell. The cells were cultured in 5% CO2 incubator for 4 days, then the 500 μg/mL RCE was added.The recombinant senescent epidermis model was cultured in the medium containing RCE for 13 days.The data of all results were compared with those of the untreated group (NT).

**5.Data statistics**

For all data, the statistical significance was assessed by the *t*-test. For the statistical results, "*" referred to the $P$ <0.05 compared with the control group, "**" referred to the $P$ <0.01 and "***" referred to $P$<0.001.

## Results & Discussion:

**Table 1 The proliferation rate of Rg Callus**

| Item | 0 d/g | 25 d/g | PC | Average PC | BR |
|---|---|---|---|---|---|
| Glacier water-1 | 0.38 | 2.16 | 5.68 | 5.96 | 7.50% |
| Glacier water -2 | 0.32 | 1.89 | 5.93 | | |
| Glacier water -3 | 0.40 | 2.54 | 6.26 | | |
| Deionized water -1 | 0.33 | 1.31 | 3.93 | 4.13 | 35.00% |
| Deionized water -2 | 0.36 | 1.72 | 4.78 | | |
| Deionized water -3 | 0.41 | 1.51 | 3.68 | | |

PC: proliferation coefficient PC= Callus quality of 25 d/ Callus quality of 0 d. BR: Browning rate BR= (The number explants of Browning/Total number explants)*100%

The results showed that using glacial water to culture Rg callus could not only increase its proliferation, but also greatly reduced the browning rate from 35.0% to 7.5% (Table.1).

**Table 2 DPPH free radical scavenging activity of RCE**

| RCE | Concentation (μg/mL) | DPPH (%) |
|---|---|---|
| Batch-1 | 0.5 | 56 |
| Batch-2 | 0.5 | 59 |
| Batch-3 | 0.5 | 60 |

It showed that RCE an effect on Anti-Oxidant.

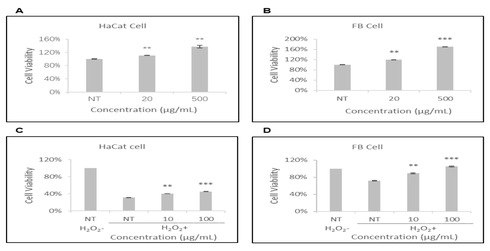


**Figure 2. Cell viability of RCE in HaCaT cells (A) and FB cells (B) for proliferation, and in HaCaT cells (C) and FB cells (D) after treated by $H_2O_2$**

It was confirmed that the RCE did not show cytotoxicity, and showed an effect on cell proliferation in HaCaT and FB cells and suggested that RCE could protect against oxidative damage

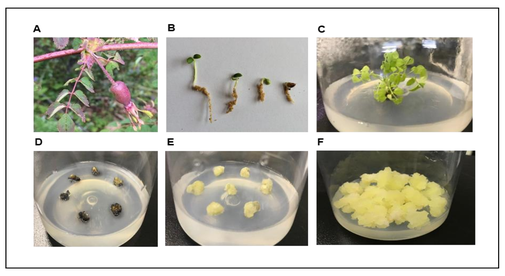


**Figure 1. Fruit pod (A), Seed germination (B), Seedling (C), Deionized water cultured callus (D), Glacier water cultured callus (E) and Callus proliferation (F) of *Rosa graciliflora***

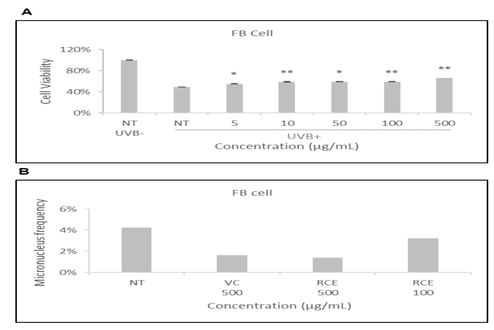


**Figure 3. Cell viability (A) and MNF (B) of RCE in FB cells after treated by UVB**

MNF:Micronucleus frequency

MNF(%)= (total number micronucleus /total number of cells) ×100

The results showed that RCE can reduce the incidence of micronuleus caused by FB in UVB and it suggested that could be used for UVB-induced damaged care for skin

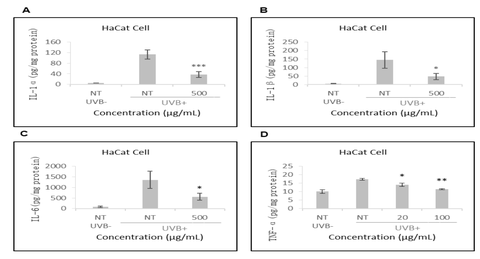


**Figure 5. Inhibitory effects of RCE on IL-1α，IL-1β, IL-6 and TNF-α production induced by UVB**

As a result of the anti-inflammatory effect, IL-1α，IL-1β, IL-6 and TNF-α production were greatly reduced by RCE under a concentration of 500 μg/mL.

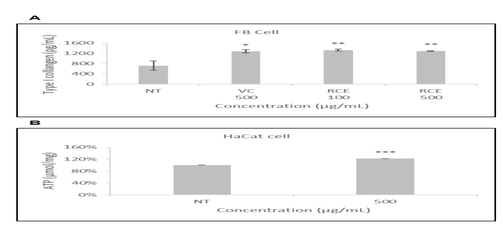


**Figure 4. Type I collagen content in FB cells (A) and ATP content in HaCat cells (B) after treated by RCE**

The results showed that RCE can increase type I collagen concent to human fibroblasts and showed that 500 μg/mL RCE could promote HaCat cell for production of ATP (Fig. 4B)

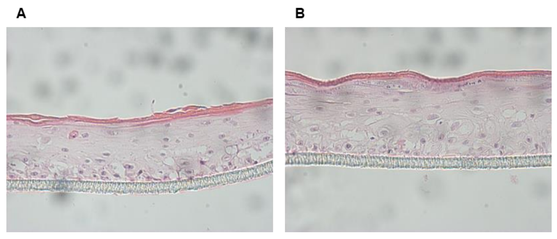


**Figure 6. HE staining in 3D skin model A (NT) and B (RCE)**

**The effects of RCE on 3D skin model**

The results of HE staining showed that a large number of vacuoles appeared in the basal layer and spinous layer of NT group (Fig.6A), and the whole structure were loose. Compared with NT group, the number of vacuolar cells in the sample group was significantly reduced, and the structure of granular layer was obvious, the cells were spindle shaped, without obvious nucleus, and the number of granules in the inner membrane was significantly increased (Fig.6B).The reconstructed 3D skin model revealed that the RCE had promosing effects on anti-aging and skin barrier strengthening.

## Conclusions:

In conclusion, the above findings contributes to the sustainable process technology developed from Rg callus, which could be a new functional cosmetic ingredient on anti-aging and skin barrier repair.

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