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Studies on Callus Culture of *Nardostachys Jatamansi* and its application in cosmetics

546

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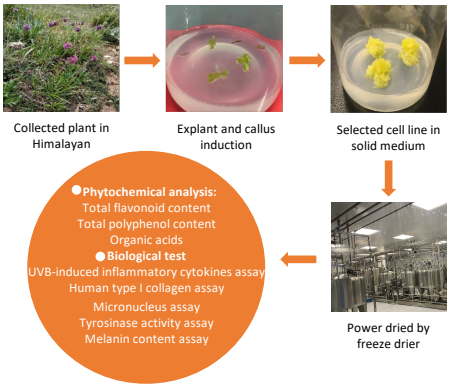
Introduction:

Plant tissue culture technology can be applied to obtain callus or regenerated plants of rare or endangered plants, which can not only obtain a large number of plant ingredients in a high efficient way, but also protect natural plant resources from destruction. Plant tissue culture extract has been successfully used in cosmetic industry because it could give abundant active ingredients with reliable quality, which have skin regeneration, anti-inflammatory, antioxidant or anti-aging effects.

Nardostachys jatamansi (D. Don) DC. (Valerianaceae family) is a typical Himalayan plant growing in alpine grasslands or gravel lands at altitudes ranging from 2600 to 5000 m. It is particularly distributed in Sichuan, Tibet, Yunnan of China and also parts of India, Nepal, Bhutan. *Nardostachys jatamansi* (NJ) is a famous typical spice plant in Tibet. It's also an important medicinal source of traditional Chinese medicine, local Tibetan medicine and Indian medicine and it has been used for the treatment of abdominal distension, pain, vomiting and inappetence as well as skin problems. For skincare and haircare, it has been reported that NJ extract showed excellent efficacy on anti-oxidation, anti-inflammatory and promoting hair growth. However, NJ resources have been seriously damaged by over-exploitation because of their great medicinal value. And it has been listed in the IUCN Red list of threatened species as Critically Endangered (CR) and one of the major CITE list plants, which must requires proper harvesting and conservation. At present, more and more scientists started to study the propagation technology of NJ, and it has been sucessfully cultivated in India and China. However, there has been little research on the chemical composition analysis and skincare benefits of extracts from NJ from tissue culture.

Therefore, the purpose of this study is to obtain sustainably source NJ callus using plant tissue culture technology, and to detect the skincare efficacy of its extract (NJCE) and evaluate its potential as a cosmetic raw material.

Materials & Methods:



Conclusions:

In this study, the callus of NJ was obtained and then the NJC was used for suspension culture and extracted to obtain the NJC extract (NJCE). The chemical component analysis showed that the NJCE was rich in flavonoids, polyphenols, and organic acids (malic acid, citric acid, fumaric acid and succinic acid). Cell assay results demonstrated that NJCE could not only decrease IL-1α, IL-1β, TNF-α and IL-6 production induced by UVB in HaCat cells, but also promote the secretion of Col-I and decrease micronucleus frequency in HDF cells. Besides, B16 cell assay revealed that NJCE could decrease the activity of tyrosinase and melanin production. These findings suggested that NJCE had potential anti-aging and whitening effect as a sustainably green raw material.

Acknowledgements:

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References:

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Results & Discussion:

The total flavonoid and total polyphenol content of NJCE were 57.64 mg/g and 25.56 mg/g, respectively. LC-MS results (Fig.2) showed that NJCE had 7.87 mg/g malic acid, 24.24 mg/g citric acid mg/g and 1.10 mg/g succinic acid (Fig. 1).

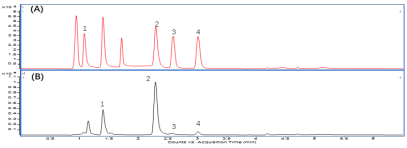

Fig. 1 LC-MS Chromatogr ams of organic acids in NJCE. A. Standard, B. NJCE

No.	Name	Content mg/g
1	Malic acid	7.87
2	Citric acid	24.24
3	Fumaric acid	0.27
4	Succinic acid	1.10
5	TFC	57.64
6	TPC	25.56

Tab. 1 The total flavonoid content (TFC), total polyphenol content (TPC) and organic acids content of NJCE

The results revealed that there was no cellular toxicity in HaCaT cells treated with 0.001%~0.05% NJCE (Fig.2 A). UVB irradiation could increase the production of IL-1α, IL-1β, TNF-α and IL-6, and the increased content of IL-1α, IL-1β, TNF-α and IL-6 were significantly inhibited by 0.001% NJCE (Fig. 2 B-E).

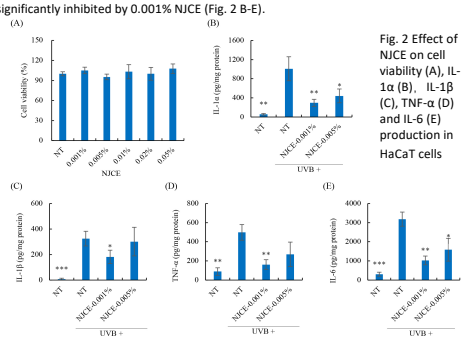

Fig. 2 Effect of NJCE on cell viability (A), IL-1α (B), IL-1β (C), TNF-α (D) and IL-6 (E) production in HaCaT cells

The cell viability of HDF cells was increased from 124% (0.01% NJCE) to 141% (0.05% NJCE) as the concentration of NJCE increasing (Fig.3 A). Col-I content of HDF cells was 620 pg/mL with not treated (NT), and 1142 pg/mL with treated by 0.05% VC. Treated with from 0.05% NJCE, the Col-I content of HDF cells was 906 pg/mL (Fig.3 B).

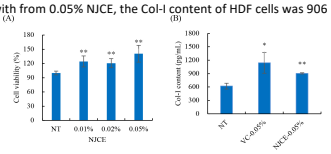

Fig. 3 Effect of NJCE on cell viability (A) and type I collagen (Col-I) content (B) production in HDF cells

Micronuclei results obtained were displayed in Table **2**. For 80 mJ/cm^2 UVB induced, the MN frequency of NT was 4.27% whereas the MN frequency decreased to 1.62% in the VC and 0.82% in the NJCE. For 160 mJ/cm^2 UVB induced, the MN frequency of NT was 10.60% while the MN frequency decreased to 2.70% in the VC and 8.48% in the NJCE.

0.01% NJCE had no effect on B16 cell viability and 0.05% NJCE slightly increased (Fig.4 A). The samples treatmented with 0.01% and 0.05% NJCE could decrease the activity of tyrosinase to 28% and 79%, respectively (Fig.4 B). Stimulation of 0.05% NJCE in B16 Cells showed a significantly reduced in melanin content by 41% compared with control (Fig.4 C).

Tab. 2 Effect of NJCE on production micronucleus (MN) frequency in HDF cells

Sample/Item	UVB-80mJ/cm2	UVB-160mJ/cm2
NT	4.27%	10.60%
VC-0.05%	1.62%	2.70%
NJCE-0.05%	0.82%	8.48%

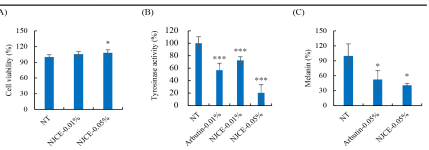

Fig. 4 Effect of NJCE on cell viability (A), melanin content (B), tyrosinase activity (C) in B16 cells