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Investigation on anti-inflammatory and whitening effects of active compounds from *Artemisia argyi*

Poster ID 296

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Introduction:

Artemisia argyi is a traditional Chinese herbal. The coumarins, flavonoids, triterpenoids, and sesquiterpenoids isolated from *Artemisia argyi* have been studied previously, and some of them showed an extremely effect on anti-inflammatory, antitumour, antimutagen and antimicrobial activity. Previous studies by my colleagues have shown that *Artemisia argyi* extract can inhibit tyrosinase activity, but its coupounds has not been thoroughly studied.

Several monomers isolated from *A. argyi* have been shown to have various biological activities. However, its whitening and anti-inflammatory functions have not yet been reported. HPLC analysis showed that eupatilin and jaceosidin were the major phenolic compounds in *A. argyi* extract[1]. Jaceosidin isolated from *A. argyi* inhibits the TPA-induced upregulation of COX-2 and MMP-9 by blocking ERK-1 and -2 phosphorylation in human breast epithelial cells[2]. At the same time, eupatilin and jaceosidin[3] inhibited the gene expressions of TNF-α and IL-4 in RBL-2H3 cells stimulated by IgE-antigen complex[4]. Moreover, the production of general reactive oxygen species (ROS) and superoxide anions during differentiation of preosteoclastic RAW 264.7 cells into osteoclasts was attenuated by scopoletin isolated from *A. argyi*[5]. Eupatilin is a powerful PPARα agonist[6], because it could increase PPARα transactivation and expression in HaCaT cells. In addition,it also suppresses IL-4 expression and and degranulation in RBL-2H3 cells[7]. Furthermore, apoptosis rate of the hypertrophic scar fibroblasts was significantly lower after adding Eupatilin, which means eupatilin inhibits the expression of PDGFβ protein in hypertrophic scars[8]. L-borneol may play an anti-inflammatory role by scavenging the photoproduct 8-OHdG, inhibiting the regulation of NF-κB by the release of IL-6, reducing IL-6 in light-damaged tissue, and promoting light-damaged wound healing[9]. A triterpene compound Lupeol[10] from *A. argyi* has wound healing activity on Swiss Albino rats. In several studies, quercetin have showed anti-inflammatory and antioxidant properties, and it is being investigated for a wide range of potential health benefits[11]. Quercetin strongly abrogates PI3K and Src kinases, mildly inhibits Akt1/2, and slightly affected PKC, p38 and ERK1/2. Naringenin[12] triggers the mitochondrial-mediated apoptosis pathway by an increased ratio of Bax/Bcl-2, subsequent release of cytochrome C, and sequential activation of caspase-3.

In this work, we have demonstrated and studied the anti-inflammatory and whitening effects of *A. argyi*. Furthermore, we have selected 9 monomeric compounds that may have high-efficiency on whitening or anti-inflammatory activities. As a result, we verified a coumarin compound scopoletin has an excellent whitening activity, while Jaceosidin, Nepetin and quercetin show the anti-inflammatory effects.

Materials & Methods:

1) Plant extract preparation

The air-dried leaves of *A. argyi* were extracted three times with 85% aqueous ethanol at 45°C assisted by ultrasound. The extracts were concentrated until no more ethanol was left. The residual solid was disperse in water and partitioned sequentially with petroleum ether, ethyl acetate. Discard petroleum ether extract phase, then left ethyl acetate extract phase.The ethyl acetate extracts were concentrated until dryness. Then dissolve the ethyl acetate phase dry matter in 80% ethanol, diluted to 1 mg/mL concentration.

2) Cytotoxicity test

The cell viability was determined using the CCK-8 assay. To investigate whether the samples exerted a cytotoxic effect on cells. B16F10 cells and RAW 264.7 mouse macrophage cells were treated with various concentrations (1-150 μg/mL) of the sampless. For comparison of minimum cytotoxic concentration of samples, the IC20 values, which represents 20% inhibitory concentration of cell viability, was determined.

3) RAW 264.7 mouse macrophage cells Nitric oxide production

Take RAW264.7 cells were seeded at a density of 1×10^{6} cells/mL into a 96-well culture plate, and stored at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, and left to grow for 24 h. Add LPS solution 1μg/mL per well to a 96-well plate, then add concentration of extract and stored at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, and left to grow for 24h. Absorbance at 450nm was detected by microplate reader.

4) B16F10 mouse melanoma cells melanin test

Take B16F10 mouse melanoma cells were seeded at a density of 2×10^{4} cells/mL into a 24-well culture plate and stored at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, and left to grow for 24 h. Add 10μL of IBMX to each well after the cells adhere to the wall. After that, add 10 μL of sample or α-arbutin (final concentration of 2mM) to each well of the sample group and positive control group respectively, and then incubate for 48-72h in a 37°C, 5% CO_2 incubator, and observe the cell growth status under a microscope. Wash the cells twice with cold PBS to stop the reaction. Add 79 μL of NaOH to each hole and heat it in an 80℃ water bath for 5-10 minutes until the melanin is dissolved. Absorbance at 450nm was detected by microplate reader.

5) Statistical Analysis

All data are expressed as mean 6 SD. Statistical significance was determined using Student's t-test and a FDR of less than 0.05 was considered statistically significant. For assays in vivo, FDR were calculated by non-parametric Mann-Whitney test.

Results & Discussion:

1) Anti-inflammatory abilities of *A. argyi* extract

After LPS induction, RAW264.7 mouse macrophages produced NO. As can be seen in Figure 1, compared with dexamethasone (200μg/mL), the NO production of RAW264.7 mouse macrophages was significantly reduced after adding *A. argyi* extract, which proved its anti-inflammatory ability. NO inhibition ability was in a dose-dependent manner.

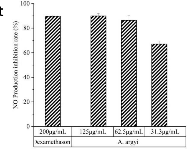

Fig.1. Effects of *A. argyi* extract on NO Production in RAW 264.7 mouse macrophage cells Macrophages Stimulated with LPS.

2) Inhibit melanin production abilities in vitro

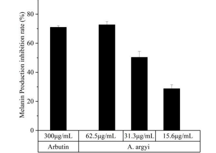

Fig.2. Effects of A. argyi extract on melanin production in B16F10 mouse melanoma cells stimulated with IBMX.

Since B16F10 mouse melanoma cells and human melanocytes are relatively close in physiology, by establishing a mouse melanoma cells line and adding IBMX, Melanin production system was established. Illustrated by the Figure 2, the ability to inhibit melanin production of *A. argyi* extract can be judged compared with α-arbutin (0.3mg/mL). Which means it has an excellent ability to inhibit melanin production, thus having whitening potential. The inhibit melanin production abilities of B16F10 cells was in a dose-dependent manner.

3) RAW 264.7 mouse macrophage cells Nitric oxide production

The ethyl acetate extract from *Artemisia argyi* was conformed to have an excellent NO inhibitory effect. such as jaceosidin, nepetin and quercetin were shown to have NO inhibitory effect. As a IgE-antigen complex, jaceosidin may be useful for protection from the PCA and itching reactions, which are IgE-mediated representative skin. Which means can be a powerful compound as an anti-allergic and soothing raw material in skin care products allergic diseases.

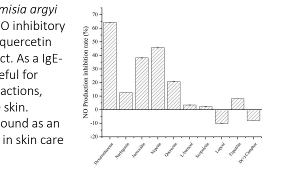

Fig.3. Effects of compounds in *A. argyi* on NO Production in RAW 264.7 mouse macrophage cells Macrophages Stimulated with LPS.

4) Inhibit melanin production abilities in vitro

Scopoletin extracted from *Artemisia argyi* was confirmed to have melanin inhibitory effect. The inhibition of scopoletin is better than that of glycyrrhizin at maximum tolerated concentration. Scopoletin may be one of the effective components . The anti-melanogenic activity of nepetin was also verified.

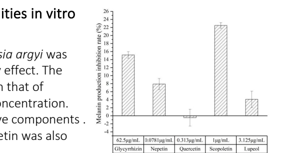

Fig.4. Effects of compounds in *A. argyi* extract on melanin production in B16F10 mouse melanoma cells stimulated with IBMX.

Although the structures of flavonoids and coumarins may have protential to be tyrosinase inhibitors, different substituents can also lead to different anti-melanogenic effect. Besides, nepetin also showed anti-melanogenic effect at lower concentrations as a flavonoid. However, whether the poor whitening effect of quercetin remains to be verified.

Above all, the extraction process of *Artemisia argyi* should tend to the enrichment of the above-mentioned active components.

Conclusions:

Artemisia argyi has a strong anti-melanin and anti-inflammatory properties. But few of its compounds have been studied in cosmetics. In this work, we studied several compounds in *Artemisia argyi* that may have anti-melanin and anti-inflammatory activities. The results confirmed that the whitening effect of *Artemisia argyi* is partly attributed to the flavonoids of nepetin, coumarin of scopoletin. At the same time, jaceosidin, nepetin, and quercetin showed high anti-inflammatory effects. The research results provided a certain basis for the compound application of *Artemisia argyi* compounds in cosmetics, and laid a foundation for the research on the whitening and anti-inflammatory mechanism of *Artemisia argyi* leaves.

Acknowledgements:

References:

1. Ha G J , Lee D S , Seung T W , et al. Anti-amnesic and Neuroprotective Effects of Artemisia argyi H. (Seomae mugwort) Extracts[J]. Korean Journal of Food Science & Technology, 2015, 47(3):380-387.
2. Min A J , Lee K W , DO-YOUNG YOON, et al. Jaceosidin, a Pharmacologically Active Flavone Derived from Artemisia argyi, Inhibits Phorbol-Ester-Induced Upregulation of COX-2 and MMP-9 by Blocking Phosphorylation of ERK-1 and -2 in Cultured Human Mammary Epithelial Cells[J]. Annals of the New York Academy of Sciences, 2010, 1095(1):458-466
3. Zhou Q , Sun L L , Jiang B , et al. Simultaneous Determination of Eupatilin and Jaceosidin in Artemisia argyi and Its Processed Products by RP-HPLC[J]. China Pharmacy, 2013.
4. Lee S H , Bae E A , Park E K , et al. Inhibitory effect of eupatilin and jaceosidin isolated from Artemisia princeps in IgE-induced hypersensitivity[J]. International Immunopharmacology, 2007, 7(13):1678-1684.
5. Lee S H . Scopoletin and scopolin isolated from Artemisia iwayomogi suppress differentiation of osteoclastic macrophage RAW 264.7 cells by scavenging reactive oxygen species.[J]. Journal of Natural Products, 2013, 76(4):615-20.
6. Yongsoo C , Yujung J , Su-Nam K . Identification of Eupatilin from Artemisia argyi as a Selective PPARα Agonist Using Affinity Selection Ultrafiltration LC-MS[J]. Molecules, 2015, 20(8):13753-13763.
7. Jung Y, et al. Eupatilin, an activator of PPARα, inhibits the development of oxazolone-induced atopic dermatitis symptoms in Balb/c mice. Biochem Biophys Res Commun. 2018 Feb 5;496(2):508-514.
8. Wu Z X, Li X, Mo Z Z, Liang J. Eupatilin Inhibits Hypertrophic Scar Proliferation Via the PDGFβ/ERK Signaling Pathway[J]. Chinese Journal of Aesthetic Medicine. Jul. 2019, 28(7): 44-47.
9. Li X T, Wang D, Pang Y X, Yang Q, Fan Z W, et al. Eeffects of L-borneol on UVB-induced Photo-damages in Skin of Balb/c Mice[J]. 2017, 19(4): 518-524.
10. B. G , Harish, and, et al. Wound healing activity and docking of glycogen-synthase-kinase-3-β-protein with isolated triterpenoid lupeol in rats[J]. Phytomedicine, 2008.
11. Rui S, Jin C, Jie D, et al. Study on Orthogonal Test Optimization of Methanol Extraction of Quercetin from Artemisia argyi Process Conditions[J]. Hubei Agricultural Sciences, 2017.
12. Arul D, et al. Naringenin (citrus flavonone) induces growth inhibition, cell cycle arrest and apoptosis in human hepatocellular carcinoma cells. Pathol Oncol Res. 2013 Oct;19(4):763-70.