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

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Abstract

Background: *Artemisia argyi* is a troditional Chinese herbal. The coumarins, flavonoids, triterpenoids, sesquiterpenoids isolated from *Artemisia argyi* has been researched previously, some of them showed an extremely effect on anti-inflammatory, antitumour, antimutagen and antimicrobial. Previous studies by my colleagues have shown that Artemisia argyi extract can inhibit tyrosinase activity, but its coupounds has not been thoroughly studied. In this study, we tried to investigate on anti-inflammatory and whitening effects of active compounds from *Artemisia argyi*.

Methods: The dried powder of *Artemisia argyi* was extracted three times with 85% aqueous ethanol at 45℃ assisted by Ultrasound. The extracts were concentrated until no more ethanol was left. The residual solid was dissolved in water and partitioned sequentially with petroleum ether, ethyl acetate and n-butanol. The anti-melanogenic and melanin inhibitory effect in B16F10 melanocytes. Anti-inflammatory abilities were determined by NO inhibitory effect in mouse macrophages RAW264.7 cells.

Results:The ethyl acetate extract from Artemisia argyi was conformed to have an excellent anti-melanogenic effect and also found that it has very good NO inhibitory effect. As a result, the compounds in ethyl acetate extract from Artemisia argyi, such as Nepetin，

Scopoletin was confirmed to have melanin inhibitory effect, such as Jaceosidin and Quercetin were shown to have NO inhibitory effect.

Conclusion:These results illustrated that Artemisia argyi compounds have anti-melanogenic and anti-inflammatory abilities. The results provide some basis for the application of compounds from Artemisia argyi in cosmetics.

Keywords: (*Artemisia argyi;* Anti-inflammatory; Anti-melanogenic; Nepetin).

Introduction.

The genus *Artemisia* is a genera of the famliy Asteraceae which consists of about 400 species of plants[1]. Numerous members of the genus *Artemisia* are as ornamental corps, as well as being medicinal and arematic plants. Many of it produce essential oils used in the cosmetics and pharmaceutical industry[2]. *Artemisia argyi (A. argyi)* is a troditional Chinese aromatic herb of the genus Artemisia. As a commonly used medicinal and edible herbal, it has been used for thousands of years in treating irregular menstruation, dyamenorrhea and nontraumatic hemorrhage[3]. Sesquiterpenoids and flavonoids in *A. argyi* were the most abundant secondary metabolites in Artemisia species.

Phytochemical researches on *A. argyi* have led to the isolation of sesquiterpenoids, triterpenoids, steroids, coumarins and flavonoids. Some of them showed an extremely effect on anti-inflammatory, immunomodulatory, antitumour, antimutagen and antimicrobial activities[4]-[6]. The organic acids, phenolic compounds, flavonoids, and methoxylated Flavonoids in *A. argyi* showed an excellent antioxidant activity, *A. argyi* has more than 45 identified antioxidants[7]. Essential oil extracted from *A. argyi* leaves decreased melanin production in B16F10 cells and showed antioxidant activity[8]. Sesquiterpene dimer from *A. argyi* could be useful for neuroprotection in inflammation-mediated neurodegenerative diseases via inhibition of microglia-mediated neuro-inflammation[9]. *A. argyi* also can be used for the treatment of bacterial infection and allergy.

Several monomers isolated from *A. argyi* have been shown to have various biological activities, But 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[10]. 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[11], at the same time Eupatilin and jaceosidin[12] inhibited the gene expressions of TNF-α and IL-4 in RBL-2H3 cells stimulated by IgE-antigen complex[13]. 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*[14]. Eupatilin is a powerful PPARα agonist[15], it could increases PPARα transactivation and expression in HaCaT cells, it also suppresses IL-4 expression and and degranulation in RBL-2H3 cells[16]. 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[17]. 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[18]. A triterpene compound Lupeol[19] 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[20]. Quercetin strongly abrogates PI3K and Src kinases, mildly inhibits Akt1/2, and slightly affected PKC, p38 and ERK1/2. Naringenin[21] 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.

Previous studies by my colleagues have done revealed that A. argyi extract can inhibit tyrosinase activity, but its mechanism of action and other potential cosmetic activity has not been thoroughly studied. In this study, 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 and Methods.

1) Materials

The *Artemisia argyi (A. argyi)* leaves were purchased from Guichen Technology Co., Ltd.(Hubei, China);B16F10 and RAW 264.7 mouse macrophage cells were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea); DMEM, heat-inactivated fetal bovine serum (FBS) were purchased from Gibco(USA); CCK-8 was purchased from Abbkine Scientific Co., Ltd(Wuhan, China); Eupatilin, Jaceosidin, Scopoletin, L-borneol, Lupeol,

Nepetin, Quercetin, Naringenin, D(+)-Camphor were purchased from nature-standard Co., Ltd(Shanghai, China) ; QuantStudio 3 was purchased from Thermo Fisher Scientific Co., Ltd(Shanghai, China)

2) 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.

3) Cell culture

RAW 264.7 mouse macrophage cells were maintained in RPMI 1640 medium which contained 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin G, and 100 mg/mL streptomycin at 37°C in a humidified incubator (5% CO_2 and 95% air).

B16F10 mouse melanoma cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100μg/mL streptomycin. Cultures were maintained in a humidifified atmosphere with 5% CO_2 at 37°C. Cells were passaged every 4 to 5 days.

4) Cytotoxicity test

The cell viability was determined using the CCK-8 assay[22]. 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 IC_{20} values, which represents 20% inhibitory concentration of cell viability, was determined.

5) 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.

6) 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℃ 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 10μL 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℃, 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 79μL 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

7) 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.

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

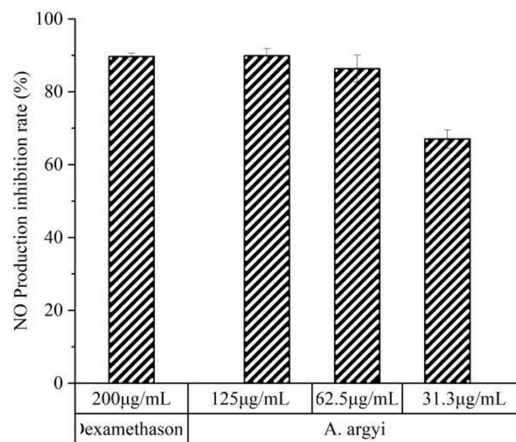

Fig.1. Effects of A. argyi extract on NO Production in RAW 264.7 mouse macrophage cells Macrophages Stimulated with LPS. The RAW264.7 mouse macrophages were treated with LPS (1μg/mL) and dexamethasone (200μg/mL) as well as three concentrations (125μg/mL, 62.5μg/mL, 31.3μg/mL) of A. argyi extracts, then NO concentration was detected by Griess assay.

A.argyi extracts with cell viability greater than 80% were used in cell culture experiments. RAW264.7 cells have a strong ability to adhere and phagocytic antigens. 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 pattern.

2) Inhibit melanin production abilities in vitro

Concentrations of *A. argyi* extracts with cell viability greater than 80% were used in cell culture experiments. 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 pattern.

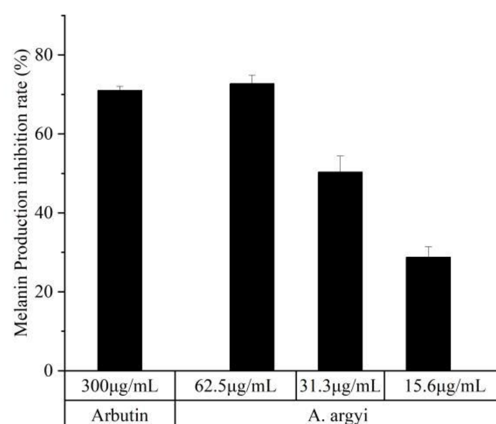

Fig.2. Effects of *A. argyi* extract on melanin production in B16F10 mouse melanoma cells stimulated with IBMX. The B16F10 mouse melanoma cells were treated with IBMX (22.2μg/mL) and α-arbutin (0.3mg/mL) as well as three concentrations (62.5μg/mL, 31.3μg/mL, 15.6μg/mL) of *A. argyi* extracts, then melanin concentration was detected. The melanin production of cells treated by IBMX was considered as 100%.

3) Cytotoxicity test

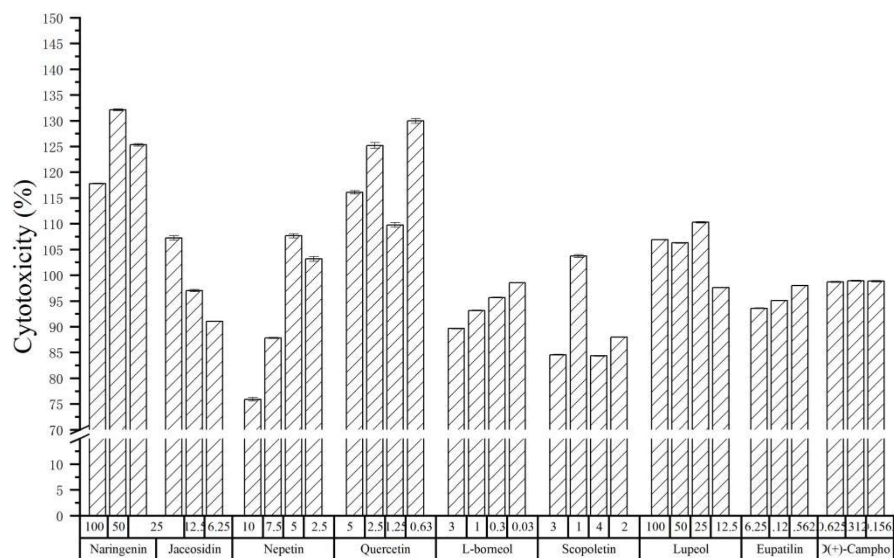

Fig.3 Cytotoxicities of compounds on RAW264.7 mouse macrophages

Cytotoxicities of compounds on RAW264.7 mouse macrophages are as we can see in Fig.3 above.We chose the experimental concentration based on the following principles. The monomer concentration used when the cell viability was the highest in the monomer cytotoxicity experiment was taken as the following experimental concentration. Table.1 shows the selected experimental concentrations.

Table.1 Monomer experiment concentration

	Naringenin	Jaceosidin	Nepetin	Quercetin	L-borneol	Scopoletin	Lupeol	Eupatilin	D(+)-Camphor
μM	100μM	25μM	5μM	5μM	0.03μM	1μM	25μM	1.5625μM	0.3125μM

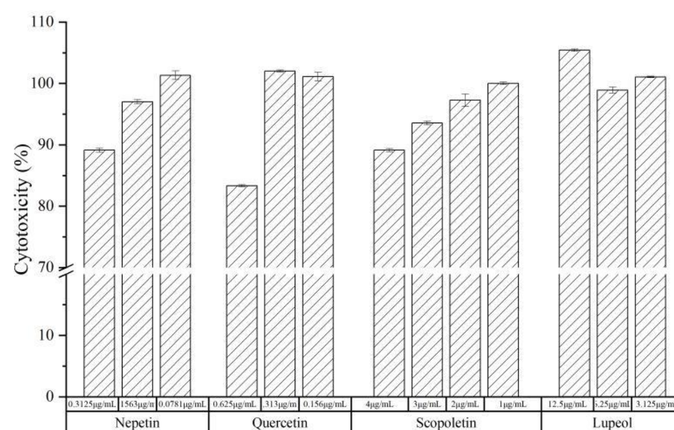

Fig.4 Cytotoxicities of compounds on B16F10 melanocyte

Cytotoxicities of compounds on B16F10 melanocyte are as we can see in Fig.4 above.We chose the experimental concentration based on the following principles. The monomer concentration used when the cell viability was the highest in the monomer cytotoxicity experiment was taken as the following experimental concentration. Table.2 shows the selected experimental concentrations.

Table.2 Monomer experiment concentration

	Nepetin	Quercetin	Scopoletin	Lupeol
μM	0.0781μg/mL	0.3125μg/mL	1μg/mL	3.125μg/mL

4) RAW 264.7 mouse macrophage cells Nitric oxide production

Main compounds Eupatilin, Jaceosidin, Scopoletin, L-borneol, Lupeol, Nepetin, Quercetin, Naringenin, D(+)-Camphor in *A. argyi* were used in cell culture experiments. After LPS induction, RAW264.7 mouse macrophages produced NO. As can be seen in Figure 5, compared with dexamethasone (200ng/mL). The NO production of RAW264.7 mouse macrophages was significantly reduced after adding Jaceosidin, Naringenin, Quercetin, showed their anti-inflammatory ability.The release of NO was slightly promoted under the action of Lupeol, D(+)-Camphor.

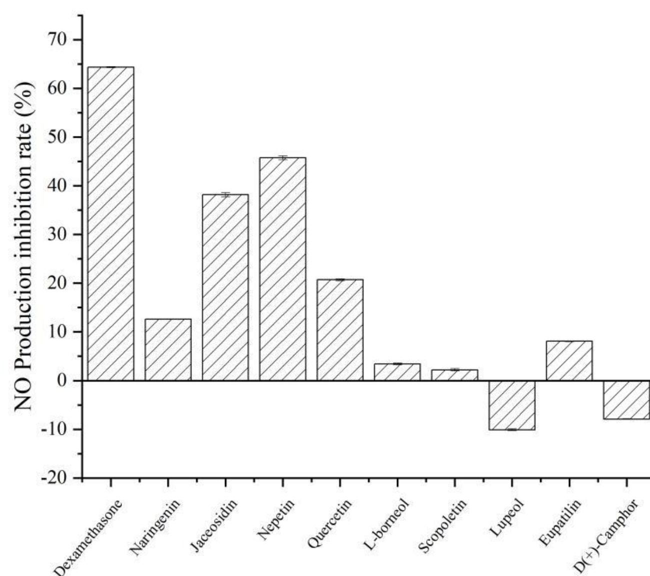

Fig.5. Effects of compounds in *A. argyi* on NO Production in RAW 264.7 mouse macrophage cells Macrophages Stimulated with LPS.

5) Inhibit melanin production abilities in vitro

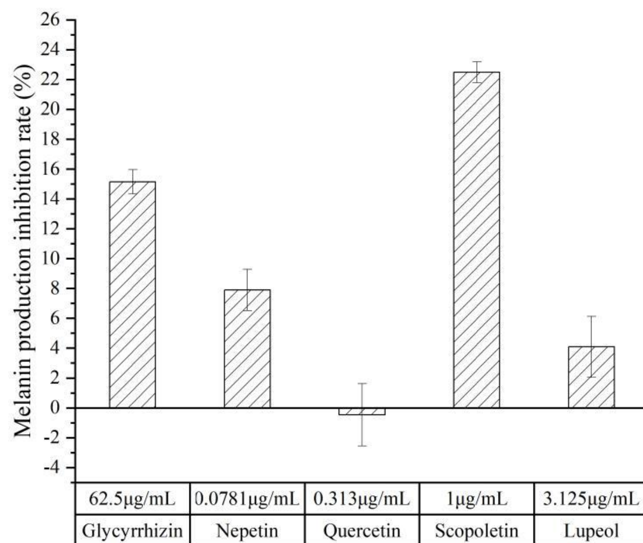

Fig.6. Effects of compounds in *A. argyi* extract on melanin production in B16F10 mouse melanoma cells stimulated with IBMX.

The B16F10 mouse melanoma cells were treated with IBMX (22.2μg/mL) and Glycyrrhizin (62.5μg/mL) was detected. The melanin production of cells treated by IBMX

was considered as 100%. Compared with Glycyrrhizin (62.5μg/mL), The compounds in we chose has somehow showed its possible ability on skin-whitening by inhibiting the Melanin production. As we can see in Fig.6, Scopoletin has a better inhibition than Glycyrrhizin at a lower concentration, Nepetin also should be noticed as a skin-whitening compound. Quercetin's melanin-suppressing ability is less than expected.

Discussion.

The ethyl acetate extract from *Artemisia argyi* was conformed to have an excellent anti-melanogenic effect. As a result, the compounds in ethyl acetate extract from *Artemisia argyi*, such as Scopoletin was confirmed to have melanin inhibitory effect. The inhibition Scopoletin has is better than Glycyrrhizin at a lower concentration. which means Scopoletin may be one of the main components of *Artemisia argyi* extract that promotes its melanin inhibitory effect. The anti-melanogenic activity of Nepetin also can be verified. Although the structures of flavonoids and coumarins may have protential to be tyrosinase inhibitors[23], different substituents can also lead to different anti-melanogenic effect. In this experiment, it was confirmed that coumarin compound Scopoletin shows anti-melanogenic effect. At the same time, Nepetin also showed anti-melanogenic effect at lower concentrations as a flavonoid. However, whether the poor whitening effect of Quercetin is based on its poor solubility or its own low activity due to its structure remains to be verified.

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.

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

Conclusion.

Artemisia argyi has a strong anti-melanin and anti-inflammatory properties. But few of its compounds have been studied in cosmetics. In this study, we studied several compounds in *Artemisia argyi* that may have anti-melanin and anti-inflammatory activities, and the

results confirmed that the whitening effect of *Artemisia argyi* is partly attributed to the flavonoids of Nepetin and coumarin of Scopoletin, and its 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.

Conflict of Interest Statement.

NONE.

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