Anti-inflammatory and anti-melanogenesis activities of flavonoids isolated from *Orthosiphon stamineus*

Poster ID：EP_179

Li Yue, Zhu Shiqiang, Bi Yongxian, Yang Xin, Xu Xiaoming, Jiang Ligang.

Introduction:

Orthosiphon stamineus(OS) is a shurb that widely used in various parts of Malaysia and Indonesia, it is often used as a tea for general health and fitness. In China, *Orthosiphon stamineus*(OS) was introduced as an ornamental plant, it began to grow on a large scale due to its unique medicinal value. It often grows in damp places under forests, and sometimes it is also found on shady flat ground. It is often used to treat diabetes, kidney and urinary disorders, high blood pressure and bone or muscular pain.

Materials & Methods:

1. Plant extraction process:

Orthosiphon stamineus leaves → ultrasonical extracted (85% ethanol) → purified by activated carbonand → purified by AB-8 macroporous

2. Measurement of melanin content:

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 24h. 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°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 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.

3. Measurement of NO content RAW 264.7 cells:

Take RAW 264.7 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 24h. 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. Inflammatory-Related and Melanogenesis-Related Gene Expression

Inflammatory-Related Gene

To evaluate whether the *Orthosiphon stamineus*(OS) extracts affected the expression of melanogenesis-related genes, such as Tyr, Trp-1, Trp-2 gene expression was examined in α-MSH melanoma cells using real-time qPCR. *Orthosiphon stamineus*(OS) had a significant effect on melanin synthesis, the gene expression can be evaluated.

Melanogenesis-Related Gene

To evaluate whether the *Orthosiphon stamineus*(OS) extracts affected the expression of inflammatory cytokines, such as NF-κB, (interleukin) IL-6, (interleukin) IL-8, (interleukin) IL-10, gene expression was examined in cells using real-time qPCR. *Orthosiphon stamineus*(OS) had a significant effect on cells, gene expression can be evaluated .

Results & Discussion:

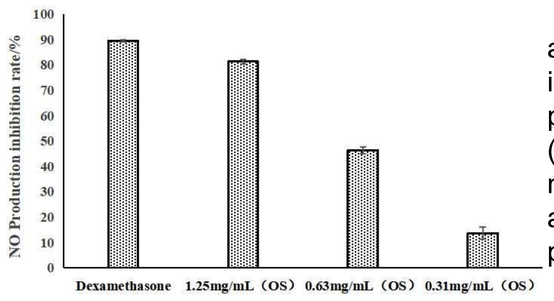
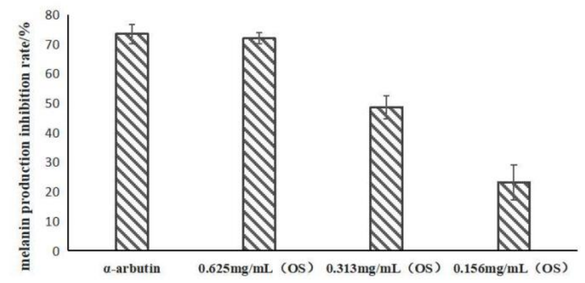


Figure1: RAW 264.7 cells have a strong ability to adhere and phagocytic antigens. After LPS induction, RAW264.7 mouse macrophages produced NO. compared with dexamethasone (200μg/mL). The NO production of RAW264.7 mouse macrophages was significantly reduced after adding *Orthosiphon stamineus*(OS) extract, which proved its anti-inflammatory ability.

Figure2: The ability to inhibit melanin production of *Orthosiphon stamineus*(OS) extract was compared with α-arbutin(0.3mg/mL). Figure shows an excellent ability of inhibiting melanin production, thus having whitening potential.



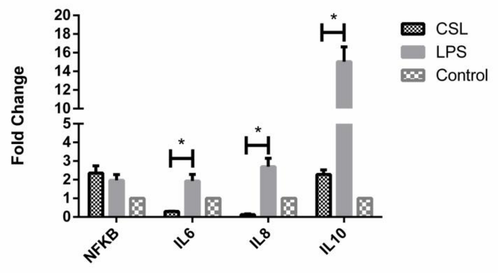
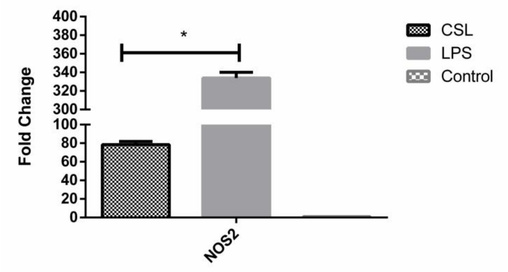


Figure3: The NF-κB, IL-6, IL-8, IL-10 gene expression were signifcantly decreased after adding *Orthosiphon stamineus*(OS) extracts. Shows an ability of anti-inflammatory by regulating the gene IL-6, IL-8, specifically IL-10.

Figure4: NOS2 gene expression were significantly decreased after adding *Orthosiphon stamineus*(OS) extracts. Shows a nice ability of Anti-inflammatory.The decrease of NOS2 shows that NO production is based on an Ca-independent situation.



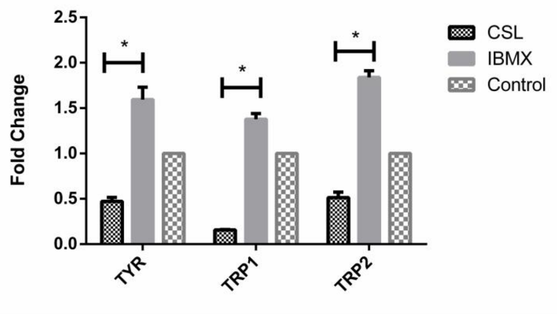


Figure5: The expression of Tyr, Trp-1, Trp-2 were decreased, which showed that *Orthosiphon stamineus* (OS) flavonoids also shows the inhibition of melanin production.The formation of melanin mainly depends on Tyr, Trp-1, Trp-2 this three enzymes.The reaction starts with the oxidation of phenylalanine and tyrosine to dopaquinone (DQ) under the catalysis of TYR, and finally produces melanin under the continuous catalysis of Trp-1 and Trp-2. Therefore *Orthosiphon stamineus* (OS) flavonoids may have potential to be a whitening ingredient.

Conclusions:

In general, the ethanol extract of *Orthosiphon stamineus* (OS) contains flavonoids after purification by AB-8 macroporous resin, which leads to its whitening and anti-inflammatory activity driven by genes proven above. Anti-inflammatory and inhibit melanin production are two common plant active effects, plants with these two functions are often added as plant actives in cosmetic formulations. These two functions suggests the potential for *Orthosiphon stamineus* (OS) to develop related active skin care products.

Aknowledgments:

References:

1. Wai-Leng, L. , & Chan, L. K. . (2004). Establishment of Orthosiphon stamineus cell suspension culture for cell growth. Plant Cell Tissue & Organ Culture, 78(2), 101-106.
2. Olah, N. K. , Radu, L. , MogoşAn, C. , Hanganu, D. , & Gocan, S. . (2003). Phytochemical and pharmacological studies on Orthosiphon stamineus benth. (lamiaceae) hydroalcoholic extracts. Journal of Pharmaceutical & Biomedical Analysis, 33(1), 117-123.
3. Hossain, M. A. , & Rahman, S. M. . (2015). Isolation and characterisation of flavonoids from the leaves of medicinal plant Orthosiphon stamineus. Arabian Journal of Chemistry, 8(2), 218-221.