

# 嘉南藥理科技大學專題研究計畫成果報告

## 利用微波萃取中草藥方法之建立

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## 摘要

過去本實驗室曾篩選常用中草藥擬開發成為美白化粧品之原料，篩選結果顯示：甘草、牡丹皮、桑白皮、桑枝等，具有抑制酪胺酸酶活性的效果，本計畫擬進一步建立以微波萃取這些中草藥之方法開發。

## 一、緒論：

從過去有關中藥材的研發中，我們可清楚的了解中藥材可用之處甚多，由主要的醫學療效到添加於飲食；甚至於化粧品當中，這些可說是廣泛的被利用，且中藥材所強調的重點，多以溫和及刺激少的理念為主。在文獻的報告中顯示，具有抗氧化及排除自由基的植物的來源有：丁香的花、桑枝的莖、牡丹皮、大黃的根莖，其抗氧活力依序為：84%、66%、81%、80%，另外依排除自由基能力而言，則為：50%、16%、22%、33%，各植物的效果還是有所差異，因此更有研究指出，若將相近的物質合成，則抗氧化的活力和排除自由基的活動力會抑制致癌物質，並減緩老化的速度，而這些也將進一步的被應用於化粧品之內，或許更能減少其他化學製品所帶來的副作用，進而達到溫和、不刺激的好效果。

## 二、材料與方法：

酪胺酸酶體外活性抑制測定方法：

將所需之中藥材磨成細粉狀，取定量之中藥材 10mg，先加 100  $\mu$ l 的乙醇(ethyl alcohol)，待稍融合後再加入 900  $\mu$ l 的水(H<sub>2</sub>O)，利用離心機以 10000rpm、五分鐘來離心，完成後取上清液 500  $\mu$ l，再加入 900  $\mu$ l 的酪胺酸(tyrosin)及 5  $\mu$ l 的酪胺酸酶(Tyrosinase)。另外在這實驗過程中需含兩組對照組，一是 100  $\mu$ l 的乙醇(ethyl alcohol)加 900  $\mu$ l 的水(H<sub>2</sub>O)，融合後取 500  $\mu$ l；加入 900  $\mu$ l 的酪胺酸(Tyrosin)及 5  $\mu$ l 的酪胺酸酶(Tyrosinase)。二是與一的步驟相同，但不須加 5  $\mu$ l 的酪胺酸酶(Tyrosinase)。以上步驟完成，將此置於室溫之下一小時後，利用分光光度計測量波長 450nm 之紫外線吸收值，最後計算酪胺酸酶體外活性抑制百分比：未加任何藥材之 450nm 紫外線吸收值減去含中藥材之 450nm 紫外線吸收值；再除以未加任何要藥材之 450nm 紫外線吸收值，在乘以百分比即可。

### 三、結果：

#### *Effect of grinding degree for ERT for tyrosinase inhibition*

In the experiments, grinding degrees of raw material were large pieces and 40, 100 screen mesh, respectively. The duration of ERT was 0-50 h. It is seen in Fig. 1. that percentage tyrosinase inhibition of licorice root aqueous extracts increases with not increase of grinding degree. However, large pieces for tyrosinase inhibition increase with the increase of duration time.

#### *Effect of duration of microwave radiation for tyrosinase inhibition*

Licorice root 10 g was extracted with water 100 ml. The duration of microwave radiation was 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 min, respectively. It is seen in Fig. 2. that the tyrosinase inhibition rate of licorice root aqueous extracts at the beginning increases with the increase of duration of microwave radiation and reaches its maximum 90.73%, then falls down slightly. So the best duration of microwave radiation is 6.5 min. Overexposure under microwave maybe causes the loss of active compound for tyrosinase inhibition.

#### *Comparison of MAE with water bath extraction, ultrasonic extraction for tyrosinase inhibition*

Figs. 3 and 4 show the effect of extraction time on percentage of tyrosinase inhibition of ultrasonic extraction and water bath extraction, respectively. The results indicate that the tyrosinase inhibition rate for ultrasonic extraction in large pieces was increase with the increase of extraction time. The traditional industrial material is often large pieces. The large pieces were used in the water bath extraction and the following experiments. Water bath 80 extraction reached higher percentage tyrosinase inhibition in 3 h. compared to Water bath 60 extraction in 40 h.

#### *Comparison of five extraction methods for nitric oxide scavenging effect and tyrosinase inhibition*

The results in Fig. 5 and Fig 6 indicate that MAE, water bath 60/80 extraction and ultrasonic extraction reach almost equal percentage scavenging effect of NO and tyrosinase inhibition for licorice root aqueous extracts in large pieces. From the experimental conditions, it can be seen that if the almost equal percentage scavenging effect or inhibition was received, ultrasonic extraction need 3 h for 77.64% scavenging rate of NO and 50.17% tyrosinase inhibition ; water bath 80 extraction needs 3 h for 77.99% scavenging rate of NO and 74.03% tyrosinase inhibition; water bath 60 extraction needs 40h for 36.44% scavenging rate of NO and 66.3% tyrosinase inhibition ;MAE only needs 6.5 min for 82.39% scavenging rate NO and 90.73% tyrosinase inhibition.

#### 四、參考文獻：

1. Biological screening of 100 plant extracts for cosmetic use (II) : anti-oxidative activity and free radical scavenging activity 19 , 1997, pp299-307
2. Mizuno, M. and Tanaka, T. Chemistry of phyto -ingredients- recent advance of crude drugs research. In *The science of plants in cosmetic*. Fragrance Press, Tokyo (1986)
3. Ames, B. N. and Saul, R. L. Oxidative DNA damage,cancer and aging. *Ann. Intern. Med.* 107, 526-45 (1987)
4. Pratt, D. E. Natural anti-oxidants from plant material.In *Phenolic compounds and their effects on Health II*,ACS symposium series 547,pp.54-71.American Chemical Society,Washington DC(1994)
5. Ohkawa,H., Oshini,N. and Yagi,K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.*Anal.Chem.*95,351-58(1979).
- 6.Fugita,Y.,Uera,I.,Morimoto,Y.,Nakajima, M., Hatano, C. and Okuda, T. Studies on inhibition mechanism of auto-oxidation by tannins and flavonoids. II. Inhibition mechanism of coffee tannin isolated from leaves of *Artemisia* species

- on lipoxygenase dependent lipid peroxidation. *Yakugaku Zasshi* 108, 129-35 (1988).
7. Ames, B. N. Dietary carcinogens and anticarcinogens-oxygen radicals and degenerative diseases. *Science*, 231, 1256-64(1983).
  8. Simic, M. G. and jovanovic, S. V. Inactivation of oxygen radicals by dietary phenolic compounds in anti-carcinogenesis. In *Food phytochemicals for cancer prevention II*, ACS Symposium Series 547, pp.20-31. American Chemical Society, Washington DC(1994).
  9. Davies, K. T. A. *Oxidative damage and repair*. Pergamon Press, New York (1991)
  10. Simic, m.G. and Bergtold, D. S. Dietary modulation of DAN damage in human. *Mutation Res.*250,17-24(1991).
  11. Hochstein, P. and Atallah, A.S. The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. *Mutat Res.*202,363-75(1988).
  12. Wttenberg ,L.W. Inhibition of carcinogenesis by minor dietary constituents. *Cancer Res.*52,2085-91s(1992).
  13. Masaki, H. Active oxygen scavenging activity in plant extracts. *Fragrance j.*8,64-74(1995).
  14. Fukuda, T. and Kitada, Y. Reactive oxygen species-scavenging effect of crude drug. *Fragrance j.*18,75-81(1995)
  15. 90年代新製品開發課題:化粧品,美白化粧品。 *Fragrance Journal* 21(1), Jan. 1993, pp.57-67
  16. 維生素C於皮膚色素沈著抑制之作用。 *Fragrance Journal* Mar. 1997, pp.55-61
  17. 日本化粧品原料市場近況。 *Fragrance Journal* 24(1), Jan. 1996, pp.64-70
  18. 美白劑開發現況及未來展望。 *Fragrance Journal* Jan. 1996, p.13-22
  19. 生藥成分的抗自由基作用。 *Fragrance Journal* 23(8), 1995, pp.75-81
  20. In vitro Effectiveness of Several Whitening Cosmetic Components in Human Melanocytes. *The Society of Cosmetic Chemists, Journal* 42, 1991, pp.361-368.