

行政院國家科學委員會專題研究計畫成果報告

計畫名稱: 杭菊之抗氧化與抗致突變活性

The antioxidative and antimutagenic activity of
Chrysanthemum morifolium Ramate

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中文摘要

本研究主要探討黃杭菊、白杭菊、甘杭菊與貢杭菊等四種杭菊(*Chrysanthemum morifolium* Ramate)品種水萃取物之抗氧化性與抗致突變活性。結果顯示四種品種之杭菊水萃取物在亞麻油酸與脂質體模擬系統作用上均具有顯著之抗氧化性, 對2 μ g生育醇亦具有抗氧化的相乘性。再者, 彼等對鼠肝均質液因氧化所生成的丙二醛亦具有很好的抑制作用。在探討四種杭菊水萃取物之抗氧化機制上, 顯示四種杭菊水萃取物之總酚類化合物含量與抗氧化活性之間有很好之相關性; 另外彼等具有還原力的性質; 對自由基與活性氧(超氧陰離子, 過氧化氫, 氫氧自由基)亦有很顯著的捕捉效果, 顯示四種杭菊水萃取物兼具有一級與二級抗氧化劑的角色。大體而言, 白杭菊在四種品種抗氧化特性上之表現最為優異。在應用上, 四種杭菊水萃取物對不同大豆油之乳化系統均具有氧化安定作用。雖然四種杭菊品種之水萃取物對IQ之誘發致突變性並無抑制作用, 然而彼等被證實對沙門氏菌(*Salmonella typhimurium*)變異株TA98均不會造成致突變性。

關鍵詞: 杭菊, 抗氧化性, 抗致突變性, 自由基, 活性氧, 大豆油。

Abstract

The antioxidant activity and antimutagenic effect of water extract of Harnng Jyur (*Chrysanthemum morifolium*

Ramat) from four varieties, including Huang Harnng Jyur (HHJ), Bai Harnng Jyur (BHJ), Gan Harnng Jyur (GHJ), and Kung Harnng Jyur (KHJ), were investigated. The water extract of the four Harnng Jyur varieties showed strong antioxidant activity in linoleic acid and liposome model systems. They enhanced the antioxidant action of Toc when each of them was mixed with Toc, indicating that each extract had synergistic effect on Toc. Moreover, each extract exhibited the inhibition on malondialdehyde formation of rat liver homogenate *in vitro*. A correlation established between the total phenolic compounds and antioxidant activity. Each extract of the four Harnng Jyur varieties showed remarkable reducing power and hydrogen-donating abilities, and they also showed effectively quenching effect on superoxide, hydrogen peroxide, and hydroxyl radical, indicating that they had effective activities as radical scavengers and as active oxygen scavengers. In other words, they act as primary antioxidants and as secondary antioxidants. BHJ was the most significant antioxidative material among the four varieties as a result of its antioxidative properties. Water extract of the four Harnng Jyur varieties can effectively retard the peroxidation of various soybean oil emulsion systems. Water extract of the four Harnng Jyur varieties showed no inhibitory effect on the mutagenicity of IQ toward *Salmonella typhimurium* TA98, however, no mutagenicity in *Salmonella typhimurium* TA98, either with or without S9 mix, was

found in the water extract of the four Harnng Jyur varieties.

Keywords: Harnng Jyur (*Chrysanthemum morifolium* Ramat), antioxidant activity, antimutagenic effect, free radical, active oxygen, and soybean oil.

前言

過去文獻指出有些抗氧化或抗致突變成分是屬於多酚類化合物，而杭菊 (*Chrysanthemum morifolium* Ramat.) 已被證實含有不少的多酚類物質，因而推測其應含有類似抗氧化或抗致突變之成分。倘若能進一步加以處理，並探討其抗氧化及抗致突變之活性，則不僅有助於機能性成分之開發，而且可提昇杭菊之經濟效益與應用價值及對人體健康之意義。因此本研究以杭菊為樣品探討其抗氧化以及其在細胞脂質氧化上的保護作用，並且解明其在油脂氧化安定性及對食品異環胺之誘致突變之抑制功能所扮演的角色。

結果與討論

一、杭菊水萃取物之抗氧化性

圖一為四種杭菊水萃取物對亞麻油酸過氧化作用之影響。由圖一 A 中可看出添加 0.125 mg 萃取物之亞麻油酸之氧化安定性與對照組之間有顯著差異 ($P < 0.05$)。再者由圖一 B 中可看出，杭菊水萃取物之抗氧化性與其濃度之添加量有密切之關係。圖二為四種樣品組對 liposome model system 有顯著的氧化安定作用，對 Toc 亦具有相乘性。為了探討四種杭菊水萃取物在生物系統的作用，本研究進一步探討彼等對鼠肝均質液的氧化安定作用。由圖三中可看出彼等對 FeCl_2 與 ascorbic acid 在促進鼠肝均質液氧化作用中具有很可觀的抑制性，由此推測其應該具有保護細胞膜免於被氧化破壞的能力。圖四即為四種杭菊水萃取物對亞麻油酸在 $\text{FeCl}_2\text{-H}_2\text{O}_2$ 氧化下均能有效抑制 MDA 之生成，藉此亦可得悉杭菊水萃取物似乎能螯合金屬離子。圖五顯示不同品

種之杭菊其受 PVP 作用後，其多酚類化合物減少之量亦有所不同，然而抗氧化性隨著多酚類含量之減少而下降。其中抗氧化活性與多酚類含量之相關係數 (r^2) 對 HHJ, BHJ, GHJ 與 KHJ 而言，分別為 0.934, 0.997, 0.758 與 0.983，顯示多酚類含量與抗氧化活性有顯著之相關性。圖六顯示還原力隨著杭菊水萃取物之添加量增加而增加，並且 10mg 的四種杭菊水萃取物其還原力大於 0.02mg 的 ascorbic acid (0.605)，由此可推測四種杭菊水萃取物應該也具有還原劑之性質。自由基是廣為熟知能夠誘發油脂過氧化作用的物質。圖七為四種杭菊水萃取物捕捉 DPPH 自由基之活性，當添加量為 0.4mg 其捕捉之大小順序為 BHJ>HHJ>KHJ>GHJ，顯示彼等能有效捕捉自由電子。圖八為四種杭菊水萃取物對 $\text{O}_2\cdot\text{H}_2\text{O}_2$ 與 $\cdot\text{OH}$ 之活性氧的捕捉活性。由圖八中可看出四種杭菊水萃取物對活性氧均具有顯著的捕捉活性。Hydroxy radical 在生物系統中是一種反應性極強的自由基 (Hochstein and Atallah, 1988)，它幾乎可以破壞細胞內之糖，氨基酸，磷脂質，DNA 鹼基與有機酸 (Aruoma, 1994)。再者， $\cdot\text{OH}$ 能將膜脂質脫掉氫原子，進而快速地使油脂產生過氧化作用。很顯然，在本研究中已證實杭菊水萃取物具有顯著之抗氧化活性，此特性似乎與彼等能捕捉活性氧有直接關係。

二、杭菊水萃取物對大豆油乳化系統之氧化安定作用

由圖九、十與十一中可看出四種杭菊由水萃取物對大豆油不同乳化系統 o/w (10:90, w/v)，o/w (50:50, w/v) 或 w/o (50:50, v/w) 與 w/o (10:90, v/w) 具有氧化安定作用。另外可看出 TBHQ 在合成抗氧化劑之中表現最顯著。因此進一步探討四種杭菊萃取物是否對 TBHQ 具有相乘作用，結果由圖十二顯示，彼等雖然對 TBHQ 不具有相乘效果，但比起單一萃取物而言卻有加強其活性的作用。

三、杭菊水萃取物之致突變性及對 IQ 化

合物之抗致突變性

本試驗針對杭菊水萃取物進行致突變性之探討，並以 IQ 化合物作為抗致突變活性探討之受質，以作為初步之安全評估，並解明彼等對 IQ 是否具有抗致突變之特性。結果顯示此四種杭菊水萃取物在 0~0.8 mg 濃度範圍內對 IQ 化合物均不具有抑制誘發致突變性的效果，至於對其它異環胺化合物或屬於不需要 S9 混合物代謝就可誘發致突變性的 direct mutagens 等，是否具有抗致突變性，則有待進一步評估。另外，表一為四種杭菊水萃取物對沙門氏菌變異株 TA98 之致突變性。由表中顯示無論是添加或不添加 S9 混合物，四種杭菊水萃取物對沙門氏菌變異株 T98 均不會造成致突變性。

結論

由本研究結果得知，四種杭菊水萃取物具有顯著的抗氧化性，並且所含之總酚類化合物含量與抗氧化活性有很好之相關性。另外亦具有顯著之還原力及對自由基與活性氧捕捉效應，這些特性是可說明彼等之抗氧化效應，並且可作為解釋彼等抗氧化作用機制。再者，四種品種之抗氧化活性之比較而言白杭菊水萃取物之抗氧化特性最為顯著。在大豆油乳化系統之應用上，彼等亦顯現出氧化安定的角色。雖然四種杭菊水萃取物對 IQ 化合物不具有抑制致突變性，然而本研究結果證實其不具有誘發致突變性，此結果不僅對燒消費者與業者具有意義，若以機能性食品的立場而言，更能彰顯杭菊在應用上的重要性。

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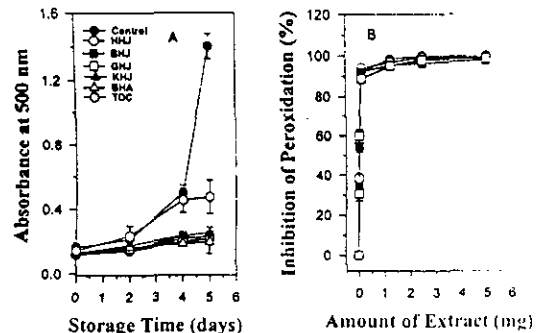
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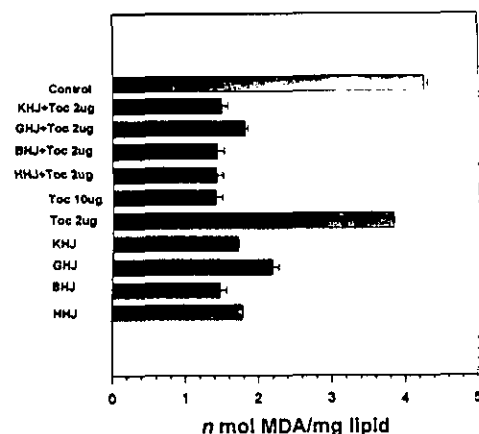
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圖一 杭菊水萃取物之抗氧化性。

Figure 1. Antioxidant activity of water extract of Hang Jyur, as measured by the thiocyanate method. A means peroxide values of linoleic acid treated with 0.125 mg water extract of Hang Jyur, and compared with 200 ppm antioxidant; B means antioxidant activity of different amounts of water extract of Hang Jyur. The percent inhibition of linoleic acid peroxidation, 100-[Abs increase of sample/Abs increase of control] × 100, was calculated to express antioxidant activity. KHJ: Huang Hang Jyur; BHJ: Bai Hang Jyur; GHJ: Gao Hang Jyur; KHJ: Kung Hang Jyur.



圖二 杭菊水萃取物與α-生育酚在脂質乳劑系統中對生有阻礙氧化性之效果作用。

Figure 2. Synergistic antioxidant activity of water extract of Hang Jyur with α-tocopherol (Toc) in a liposome model system, as measured by the thiobarbituric acid method. Lipid peroxidation was induced by FeCl₂ plus ascorbic acid, and compared with 2.0 μg Toc. TBARS: thiobarbituric acid reactive substances; MDA: malondialdehyde; other abbreviations as showed in Figure 1.

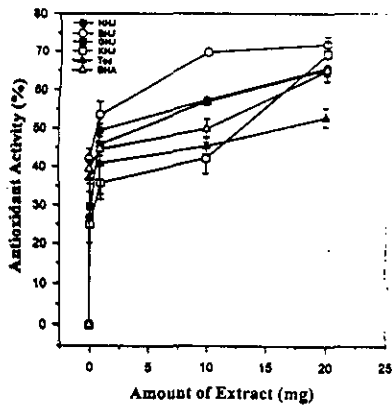


Figure 2. Laboratory effects of different amounts of water extract of Hanyang Jyur on malonaldehyde formation in rat liver homogenate induced by FeCl₂-ascorbic acid in vitro. The percent inhibition of liver tissue peroxidation, 100-[(Abs of sample/Abs of control)], was calculated to express antioxidant activity. THQ: tocopherol, other abbreviations as shown in Figure 1.

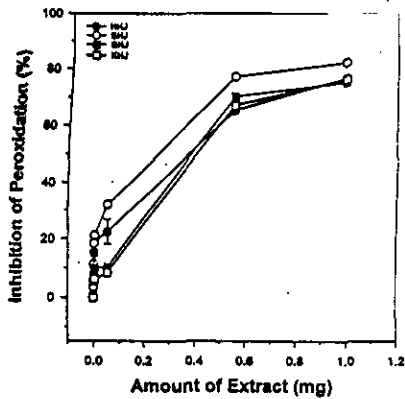


Figure 3. The effect of different amounts of water extract of Hanyang Jyur on malonaldehyde formation from linoleic acid oxidized by FeCl₂-H₂O₂. Abbreviations as shown in Figure 1.

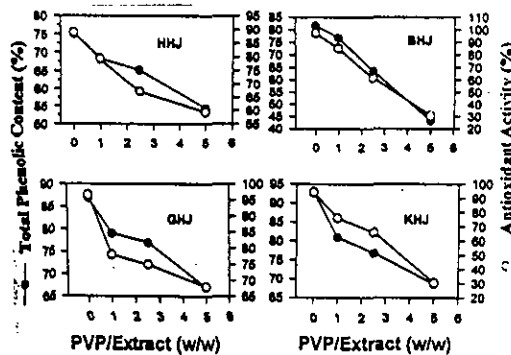


Figure 4. The correlation between total phenolic content and antioxidant activity of water extract of Hanyang Jyur. The antioxidant activity was determined by the thiocyanate method, and inhibition of peroxidation of linoleic acid and peroxidation is the same as Figure 1. PVP: polyvinylpyrrolidone; other abbreviations as shown in Figure 1.

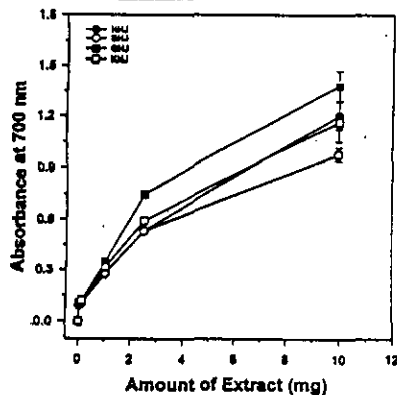


Figure 5. Laboratory effect of different amounts of water extract of Hanyang Jyur. Abbreviations as shown in Figure 1.

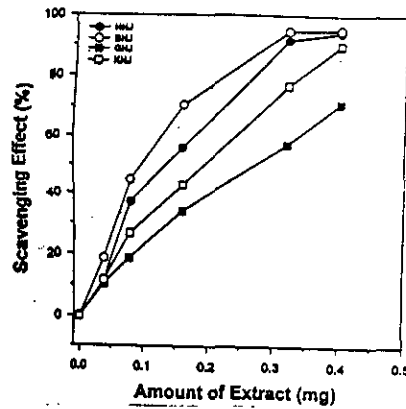


Figure 6. Scavenging effect of different amounts of water extract of Hanyang Jyur on DPPH free radical. Abbreviations as shown in Figure 1.

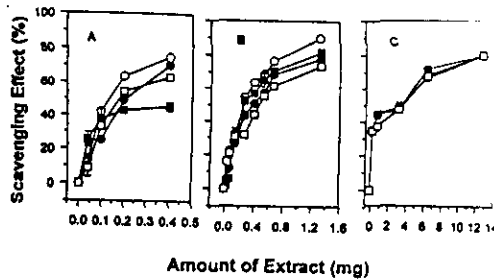


Figure 7. Scavenging effect of different amounts of water extract of Hanyang Jyur on superoxide (A), hydrogen peroxide (B), and hydroxyl radical (C). Abbreviations as shown in Figure 1.

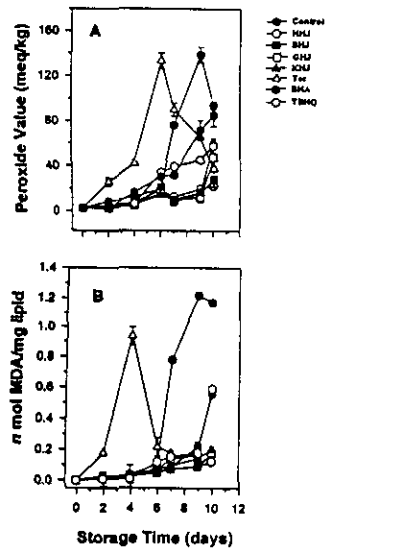


Figure 8. Antioxidative action of the water extract of the Hanyang Jyur in soybean oil/w (10:90, w/w) emulsion stored at 60 °C, as measured by peroxide value (A) and by TBARS (B). HJH: Hanyang Jyur; BHJ: Bai Hanyang Jyur; GHJ: Gu Hanyang Jyur; KHJ: Xiang Hanyang Jyur; THQ: tocopherol; BHA: butylated hydroxyanisole; TBHQ: tertiary butyl hydroquinone. The concentration of each sample was 200 ppm.

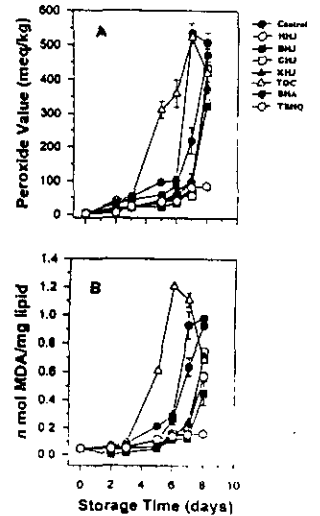


Figure 9. Antioxidative action of the water extract of the Hanyang Jyur in soybean oil/w (50:50, w/w) emulsion stored at 60 °C, as measured by peroxide value (A) and by TBARS (B). Abbreviations as in Figure 1. The concentration of each sample was 200 ppm.

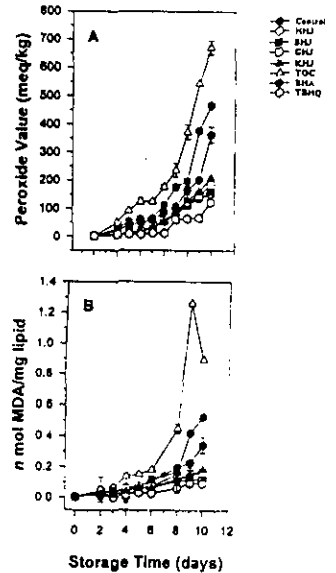


Figure 10. Antioxidative action of the water extract of the Hanyang Jyur in soybean oil/w (10:90, w/w) emulsion stored at 60 °C, as measured by peroxide value (A) and by TBARS (B). Abbreviations as in Figure 1. The concentration of each sample was 200 ppm.

Table 1. Inaugurancy of water extract of four Hanyang Jyur varieties towards S. typhimurium TAY9 either with or without SP mix.

Sample (mg/plate)	H ₂ O revertants/plate			
	HJH ^a	BHJ	GHJ	KHJ
-S ^b	-59	-59	-59	-59
0.04	44.52 ^c	39.22	47.62	43.22
0.7	46.22	39.22	42.84	45.22
0.8	44.26	46.22	49.22	45.22
SR ^d	43.22	51.22	45.22	51.22

^aAbbreviations as shown in Figure 1.

^bSpontaneous revertants (SR) were obtained without water extract of four Hanyang Jyur varieties.

^cValues are mean±standard deviation of three replicate analyses.

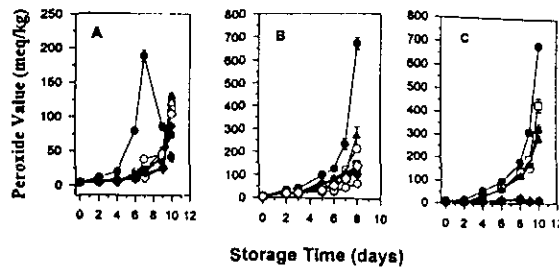


Figure 11. Synergistic antioxidative action of the water extract of the Hanyang Jyur with tertiary butyl hydroquinone (TBHQ) in soybean oil/w (10:90, w/w) (A), oil/w (50:50, w/w) (or oil/w, 50:50, w/w) (B), oil/w (10:90, w/w) (C) emulsions stored at 60 °C, as measured by peroxide value. Abbreviations as in Figure 1.