Analyzing of Major Active Luteolin in Vernonia Cinerea

Guan-Rui Lai and Chiao-Song Wu

***Abstract*—This study investigates the extraction of the Vernonia cinerea using supercritical carbon dioxide extraction under the following conditions pressure at 300, 400 and 500 bar, temperatures at 313, 323 and 333 K, CO_2 flow rate at 2 and 3 L/min. The best operation of extraction is under 500 bar, 333 K and 3 L/min CO_2 flow rate. The reverse-phase high performance liquid chromatography method was developed for determining major active components of Vernonia cinerea. We dissolve the sample with methanol and analyzed by using a MetaChem C18-A(250 × 4.6m, 5μ) column with a gradient elution composed of ultra-pure water and acetonitrile. The wavelength of UV detector was set at 280 nm and the flow-rate was set at 1 mL/min. Under this condition, the ingredients of Vernonia cinerea can be detected.**

***Index Terms*—Vernonia cinerea, supercritical carbon dioxide, luteolin, flavonoids.**

I. Introduction

Organic solvent is usually used to extract product. But organic solvent must be separated out and therefore the procedure is quite complicated.

Extraction of biomass by Press method is easy to destroy the structure and thus affect the quality of the raw materials. The most commonly used in cellulite fruit extraction of essential oil, the extraction yield is good, the procedure is simple, but the extract containing a lot of impurities must be made through the separation and purification, only relatively pure product make it suitable for high-value products.

Steam distillation is most commonly used for oil extraction, the hot steam extract the essential oils of the plant, but if the essential oil has the thermal unstable property. The other drawback of steam distillation is that only some of active composition is extracted. Substance exist as gas, liquid or solid phase, but when the temperature and pressure above its critical temperature and critical pressure, it enters the so-called supercritical fluid state, shown in Fig. 1. When the temperature is below the critical temperature, It have obvious interface between the gas-liquid phase, but the critical point is reached, this interface disappear. The physical properties of the supercritical fluid are between the gas and liquid. The density of supercritical fluid is near to the density of liquid. So that the dissolution power of supercritical fluid is higher than that of gas. The viscosity of supercritical fluid is near to that of the gas, so the conveying power in the supercritical fluid is higher than that in liquid. The diffusion coefficient of supercritical fluid is 10 to 100 times higher than that of liquid.

Supercritical fluid extraction is a process with no pollution, no toxic solvent, no organic residues. Carbon dioxide can be easily removed in supercritical fluid extraction.

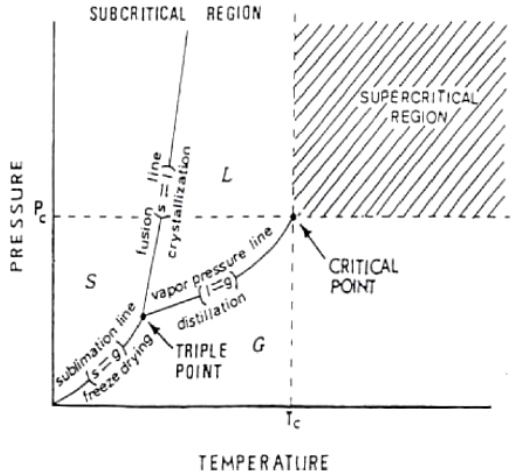

Fig. 1. The phase diagram of the substance [1].

II. Vernonia Cinerea

Vernonia cinerea is a perennial grass plants. Its stems with vertical stripes is erected, The height of Vernonia cinerea is forty to one hundred centimeters. The shape of leaves are ovate or oval, the leaves have three to seven centimeters long and one to three centimeters wide.

Vernonia cinerea is distributed in mainland China, Fujian, Guangdong, Guangxi, Jiangxi, Hunan, Sichuan, Yunnan and Vietnam, Myanmar, India, the Philippines, Australia, Africa and other places. It is distributed in Taiwan among the plains, the waterfront and the low altitude of the mountain. The stems of Vernonia cinerea are cooked or fried [2].

A. The Physiologically Active

Vernonia cinerea is picked in late spring to winter and is collected after the removal of soil and then dried for use.The whole plant of Vernonia cinerea is used as medicine .Its flavors are Cool or slightly cold, bitter, slightly sweet slightly acrid. The effectiveness of Vernonia cinerea is free to fever, to stop bleeding, the anti-inflammatory, detoxification, swelling.

Flavonoids categories (Flavonoids) are widely distributed in nature. It is a type of important natural organic compounds. The ionization states of Flavonoids exist in plants. The majority type of Flavonoids is combined with sugar into glycosides type. The flavonoids categories refer to the two benzene rings through mutual associates with

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three carbon chain compounds. Natural flavonoid classified on the mother nucleus of compounds often contain substituents such as a hydroxyl group, a methoxy group, a methyl group, an abnormal pentenyl group, etc. According to their structural characteristics of Flavonoids , can be divided into the flavonoids (Flavone), flavonols (Flavonol), Isoflavones (Isoflavone) and flavanones of alcohol (Flavanol).Flavonoid group of compounds is often exited as crystalline solid majority or minority (such as flavonoid glycoside analog) powder. Flavonoid types of six Compounds of different structure have different solubility[3]. The flavonoid glycosides types is soluble in methanol and ethanol polar organic Solvent, due to the molecules and intermolecular permutation closely. It is difficult to dissolve in water. Flavonols, such as a flat-type molecule, due to stuffing closely intermolecular attraction is large, it is difficult to dissolve in the water. Flavonoid types of plant ingredients with a wide range of different plant components may have different physiological activity and pharmacodynamics role[4]. Currently there are number of thousands of flavonoid group of compounds is separated, including domestic and foreign scholars. From Vernonia cinerea carved from the flavonoid group of compounds identified active ingredients, such as: apigenin (Apigein), luteolin (Luteolin), Quercetin (Quercetin) [5]. Luteolin has a role on the cardiovascular system, central nervous system function, Enhanced cell immunity,anti-cancer and anti-tumor function of a variety of pharmacological.

B. Supercritical Extraction Technology

The extraction of Supercritical fluid carbon dioxide is better than traditional extraction. The component of extract depends on the temperature and pressure. Changing the temperature and pressure can vary the component solubility of extract in supercritical carbon dioxide. The temperature in the supercritical fluid extraction process is low. The active ingredient in herb is not destroyed. So the heat sensitive active ingredient can be extracted.

Supercritical fluid carbon dioxide extraction is non-polluting, non-toxic, non-organic residues and environmentally-friendly, Supercritical fluid carbon dioxide extraction make the process of extraction and solvent removal into one simplified operation and improve extraction efficiency. Supercritical carbon dioxide extraction technology is a room temperature extraction, no damage to the heat-sensitive substances. Have a great impact on the destructive effect of high temperature food nutrition, especially vitamins. Supercritical carbon dioxide extraction is a process without the participation of oxygen and light and with stable product quality [6].

III. Experimental Method

Vernonia cinerea is ground by artificial method. The ground powder size was controlled within 1mm. Then ground power was placed in an oven at 60 ℃ and was dried for 3 hours. Water was removed before extraction. Water in herb will affect the results of the experimental data. After drying, 10 g of Vernonia cinerea powder was put into the extraction tube. The volume of extraction tube is 100 mL. The extraction tube was put into the thermostatic chamber. The temperature of thermostatic chamber was set at 313, 323 and 333 k.

Carbon dioxide comes from CO_2 cylinder. The temperature of carbon dioxide condenser was set at 1 ^{0}C. Carbon dioxide from condenser was pumped into the extraction tube by high pressure pump. The pressure of high pressure pump was set to 300, 400 and 500 bar.

The extraction tube was controlled in constant temperature and pressure. The time of static extraction was set at 30 minute. After the static extraction, dynamic extraction is proceeding. The flow rate of carbon dioxide in dynamic extraction was set at 2 and 3L/min, and the extract of herb was collected every thirty minutes and the weight of extract. The reverse-phase high performance liquid chromatography was used to determine major active components of Vernonia cinerea from supercritical carbon dioxide extraction. We extracted the sample with methanol and analyzed by using a metachem C18-A(250×4.6mm, 5 μ) column with a gradient Elution composed of ultra-pure water and acetonitrile. The wavelength of UV detector was set at 280nm and the flow-rate was set at 1mL/min. Under this condition, the ingredients of Vernonia cinerea can be detected [2].

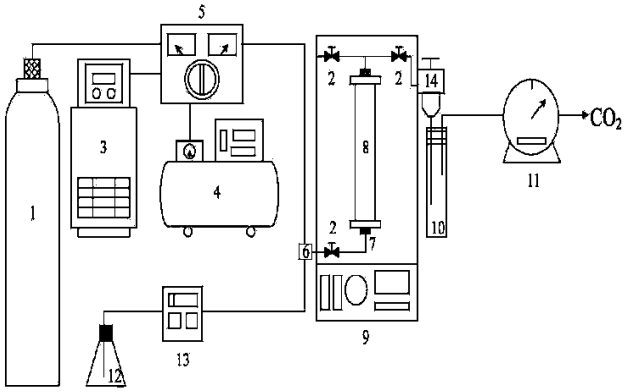

Fig. 2. Schematic diagram of the supercritical extraction. 1:CO_2 Tank, 2:Control Valve, 3:Cooler,4:Air Compressor, 5:Compressor Pressure transducer and Controler, 6:Mixed Block, 7:Filter, 8:Extracted Tube, 9:Heater and Thermometer, 10:Sample Collect Bottle, 11:Wet Test Meter, 12:Co-solvent Bottle, 13:Liquid Pump, 14:Micro Valve.

IV. Results and Discussion

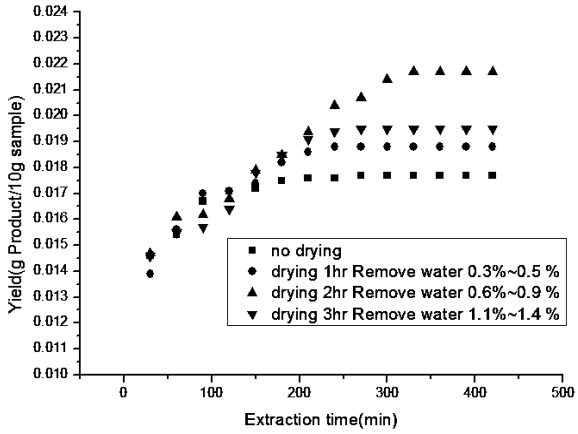

Fig. 3. The extraction curve of Vernonia cinerea at pressure 500 bar, temperature 333 K, flow 2 L/min under the drying time.

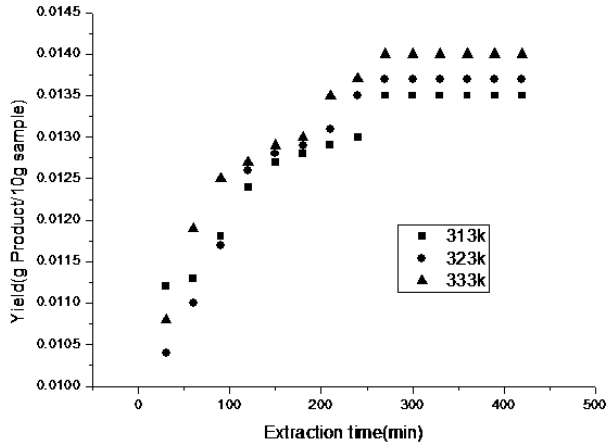

Fig. 4. Pressure 300 bar flow 2 L/min and not under the same temperature extraction curve.

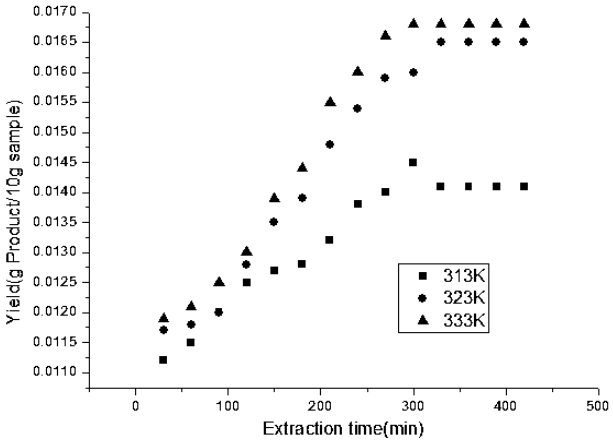

Fig. 5. Pressure 400 bar, the flow 2 L/min different temperature extraction curve.

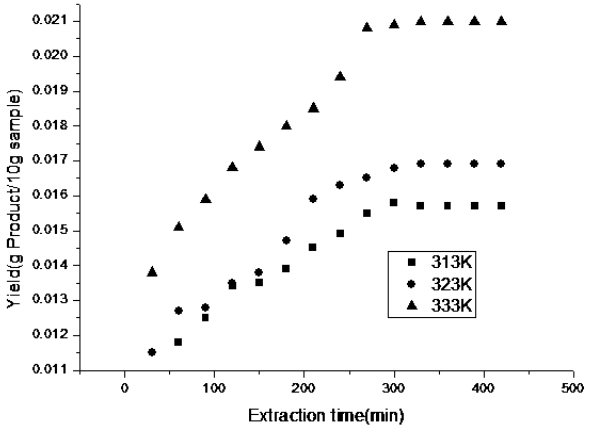

Fig. 6. Pressure 500 bar, the extraction flow 2L/min different temperature curve.

If the samples in supercritical fluid extraction process have moisture, water may form ice to cause instrument blocked. Thus we remove excess moisture from samples.

The experimental results of Vernonia cinerea extraction at300 K, 500 bar, 3 L/min CO_2 flow rate are shown in Fig. 3. The drying time of largest yield in extraction is 2 hours. When the pressure and CO_2 flow rate are fixed, we change the extraction temperature. Two factors will affect the yield of extraction. When the temperature increase, the carbon dioxide density is decreased and the solute solubility in the carbon dioxide fluid is reduced. Another factor is that when the extraction temperature increases, the vapor pressure of solute increases. The solute dissolving power increased. In this study, the yield of extraction increase with increasing temperature as shown in Fig. 4 - Fig. 6. In the fixed temperature and pressure, it will increase the yield of extraction with increasing CO_2 flow rate as shown in Fig.6. The mass transfer resistance will decrease with increasing CO_2 flow rate. If we fixed the temperature and the CO_2 flow rate. It will increase the yield of extraction with increasing extraction pressure as shown in Fig. 7. In high extraction pressure, the density of supercritical fluid will increase and the solubility of solute will increase.

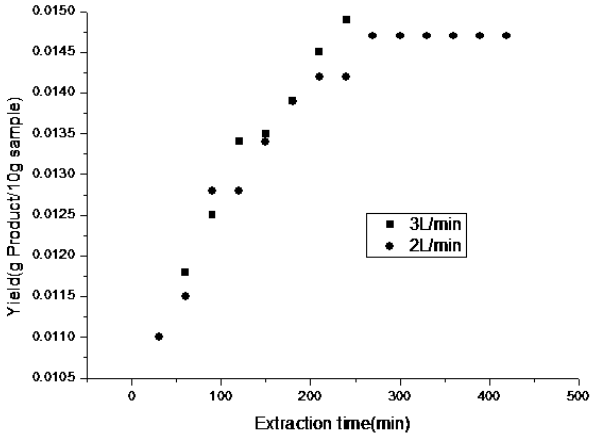

Fig. 7. Pressure 500 bar, temperature of 333 K, not the same flow extraction curve.

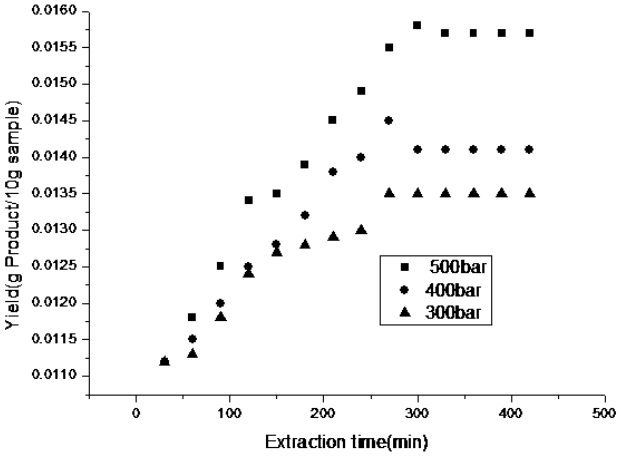

Fig. 8. Temperature of 333 K, flow 3 L/min and not under the same pressure extraction curve.

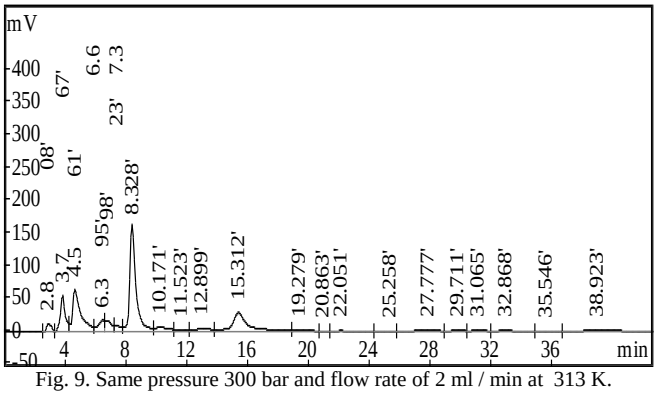

Fig. 9. Same pressure 300 bar and flow rate of 2 ml / min at 313 K.

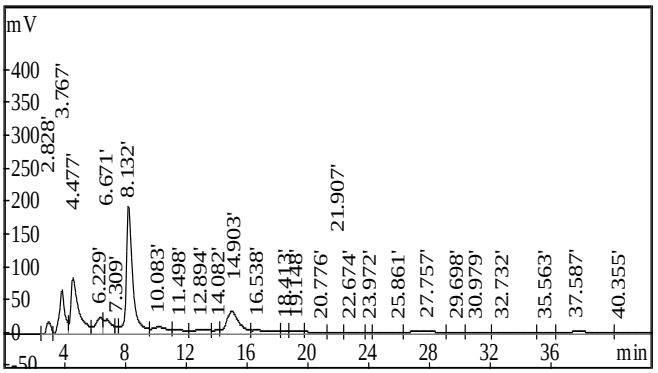

Fig. 10. Same pressure 300 bar and flow rate of 2 ml / min at 323 K.

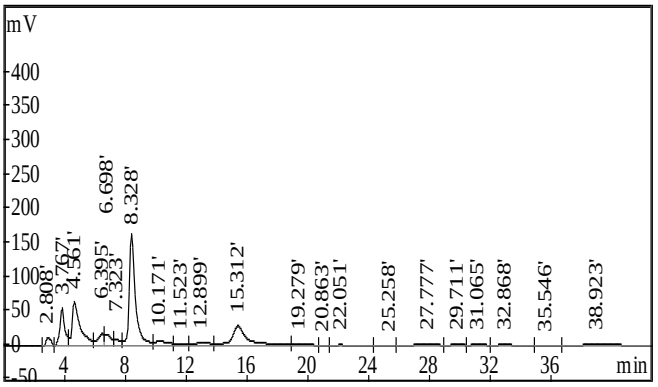

Fig. 11. Same pressure 300 bar and flow rate of 2 ml / min at 333 K.

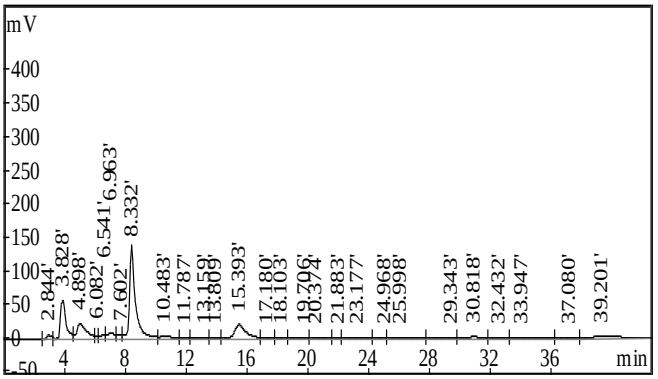

Fig. 12. A temperature of 333 k, flow 3 ml / min the same under 300 bar.

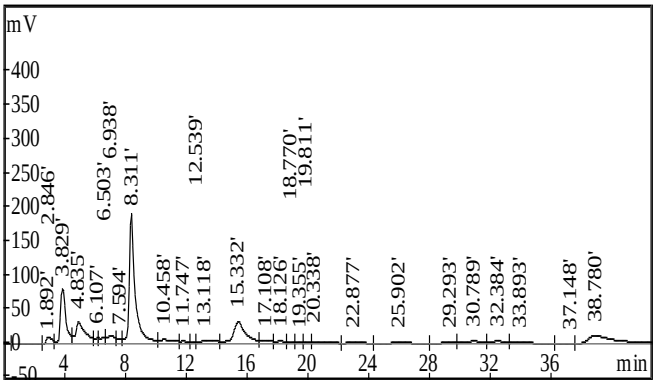

Fig. 13. A temperature of 333 k, flow 3 ml / min the same under 400 bar.

Fig. 9 - Fig. 11 is at the pressure300bar and flow rate 2 mL / min at different temperature were compared, the retention time of Luteolin in HPLC is about 8.4min. Fig. 9 - Fig. 11 shows that the content of Luteolin will be almost same between 313K and 333K. Fig. 12 – Fig. 14 is at the temperature 333K and flow rate 3 mL / min at different pressure were compared, Fig.12 - Fig. 14 shows that in 400bar the content of Luteolin will be larger than 300bar and 500bar. Fig. 15 is the HPLC figure of standard Luteolin. The retention time of HPLC column is about 10 minutes.

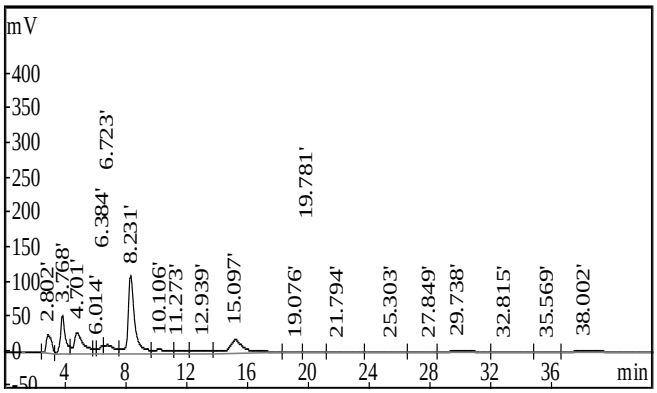

Fig. 14. A temperature of 333 k, flow 3 ml / min the same under 500 bar.

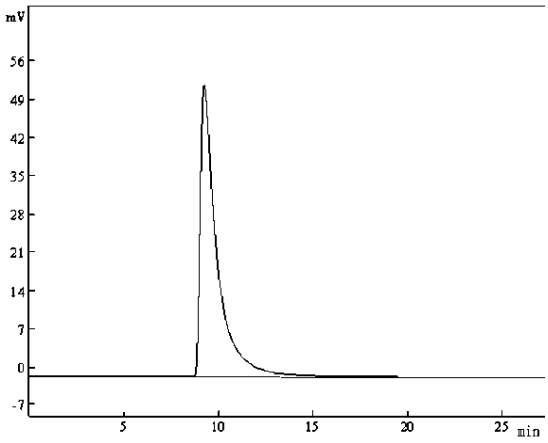

Fig. 15. Luteolin component of the standard retention time.

V. Conclusion

The traditional extraction used a large amount of organic solvent. Organic solvent is pollution liquid. It needs much energy and money to separated organic solvent from extract. It is not suitable for heat sensitive herbs. In this study, the best condition of extraction is under extraction pressure 500 bar, extraction temperature 333 K, the CO_2 flow rate 3 L/min.

References

[1] R. M. Smith, "Supercritical fluids in separation science - the dreams, the realityand the future," *Journal of Chromatography A*, vol. 856, pp. 83-115, 1999.

[2] H.-S. Lai, "Interest with supercritical active ingredients and analysis of fluid extraction Fund Yinhua," *Ming Chi University of Science and Technology*, Institute of Chemical Engineering and Materials Master thesis, 2008.

[3] *Taiwan Medicinal Plant Resources Directory of Health*, Department of Traditional Chinese Medicine Committees the typhoid grass, pp. 468, 2003.

[4] P. L. Li and D. J. Hui, "Determination and assay validation of luteolin andapigenin in human urine after oral administration of tablet of in human urineafter oral administration of tablet of Chrysanthemum morifolium extract by HPLC," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 41, pp. 261–265, 2006.

[5] H. P. Lee, "Analytical, microwave extraction of total flavonoids," *Bitter gourd Yeh*, Shaoyang University, vol. 4, pp. 86-89, 2007.

[6] N. C. Cook and S. Samman, "Flavonoids-Chemistry metabolism cardioprotective effects and dietary sources," *Journal of Nutritional Biochemistry*, vol. 7, pp. 66-76, 1996.

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