HOT PRESSURIZED WATER EXTRACTIONS OF FREE RADICAL SCAVENGING COMPOUNDS FROM TAIWAN *"DIOSCOREA ALATA"*

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This study investigated the scavenging-activity on the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals by the extracts of both meet and peel portions of three Taiwan *Dioscorea alata* (Yams), individually. The EC_{50} value, defined as the concentrations of free radical scavenging compounds sufficiently decreased DPPH absorption down to 50% of the original level, was detected for each extract. Our results found that all peel portions have better effect on scavenging DPPH free radical activity than meat portions, especially from the ethyl acetate partition of the Tainung #2 Yam. Its EC_{50} value (14.5 μg/ml) was even lower than that of ascorbic acid (21.4 μg/ml) used as the reference antioxidant. Furthermore, a few semi-continuous hot pressurized water extractions showed positive results to extract the scavenging-activity compounds out from the Taiwan yams. The saturated extraction condition was found at 120 °C and 300 psig, when 10 mL/min water flow rate used to extract 70 gram solid peels.

INTRODUCTION

In vitro experiments have demonstrated that secondary metabolite compounds of higher plants are able to against oxidative damages by inhibiting or quenching free radicals and reactive oxygen species [1]. Free radicals play an important role as causative agents in a variety of chronic diseases, such as cancer, aging [2, 3], and Alzheimer disease [4]. Several kinds of natural components in plants possess antioxidant activities, like anthocyanin [5], phenolic compounds [6, 7, 8], water extract of propolis [9], just to name a few. The tuberous rhizophors of many species from the family of *Dioscorea* were used extensively as a traditional crude drug and food in Chinese society, and still are today. A few recent literatures reported that *Dioscorea* (Yams) possess antioxidant, antifungal [11], antimutagenic compounds such as dioscorine, dioscorin, dioscin, dioscorea-mucilage, (+)-ß-Eudesmol, and Paeonol [10]. Those findings have brought worldwide attentions on *Dioscorea*, however, most researches emphasize on *Dioscorea* bioavailability, not a few on process development in extracting active compounds from yams. Hot pressurized fluids extraction showed a few

successful applications on natural plant materials [12, 13]. This study examined the feasibility to extract free radical scavenging compounds from Taiwan yams by using hot pressurized water extraction.

MATERIALS AND METHODS

Three species of Dioscorea *alata* tubers (Darsan, Tainung #2, and *Purpurea-Roxb*) were furnished by Mingjian Shiang Farmers' Association, Taiwan. All dried crude bodies were divided into meat and peel portions. A high-speed mixer grinded them down to the standard size of # 40 mesh (around 420 μm) and stored in a vacuum container before use. The powders were extracted in a 170 mL Soxhlet extractor with 95 % ethanol under reflux for 3 hours, each sample was extracted twice by fresh ethanol at boiling point, and then the extract solution was combined and concentrated under vacuum to remove the ethanol to obtain the aqueous ethanol extracts. The aqueous ethanol extract of each sample was further isolated into five fractions using liquid-liquid partitions individually with hexane, chloroform, ethyl acetate and *n*-butanol. The six fractions were ready to treat for the detection of scavenging activity on DPPH free radical.

Detecting DPPH scavenging activity was carried out according to the method of Mensor et al. [14] with slight modification. Sock solutions of each fraction diluted to five different concentrations in ethanol and reacted with DPPH ethanol solution (2×10^{-4} M) at room temperature. Stable attains after 30 minutes, the absorption value was measured and recorded by a U-3000 UV/Vis spectrometer (Hitachi, Japan) at 517 nm. EC_{50} value was calculated by a liner regression of the plot that the abscissa represented the concentration of extracts and the ordinate showed the absorption value of reacted solution averaged from two individual tests, and defined as the concentration of sample sufficient to reduce down to 50% of the maximum absorption value estimated in the blank test.

Hot pressurized water (HPW) extraction was performed in a one-liter stirred extractor. The extraction system consisted of one JASCO model PU-1580 intelligent pump (JASCO, Tokyo, JP) delivering water at constant flow rate (10 mL/min) to a PARR 4520 autoclave linked with a PARR 4842 temperature controller (PARR, Illinois, USA). In order to ensure the water was in the liquid state at all of the temperatures tested, a SS-31RF2 regulating valve was placed at the outlet of the autoclave to maintain the system pressure at 300 psig. The autoclave was initially filled with a certain amount of yam peel and water, then mounted vertically and pumped water flowing from bottom to top.

For studying the effect of temperatures, the extractor was preheating to the required temperature before pumping water. When the system achieved the required pressure (300 psig), the extraction time was set to zero. For doing solid loading experiments, the system temperature was selected at 120 according to the best result of temperature effect experiments.

The solid loadings (solid weight divided by flow rate) were 5, 7, 10, and 13 g. min/mL, and all extractions were carried out for 3 hours. After the on-time sampling, the scavenging ratio (SR) of the extract was determined by the following equation to represent the percentage of DPPH free radical being scavenged.

$$SR(\%) = \frac{(ABS_{blank} - ABS_{reacted})}{ABS_{blank}} \times 100\%$$

RESULTS AND DISCUSSION

Table 1 listed the EC_{50} values of six partitions produced from liquid-liquid extractions for two portions of three Taiwan yams. Data indicated that the last aqueous partition did not show any scavenging effect on DPPH free radical after liquid-liquid extractions. All of the effective antioxidants were totally extracted by five organic solvents from aqueous phase. It revealed that the antioxidants are soluble easier in organic solvents than in water. Furthermore, antioxidants are more easily dissolved in mid to high polarity solvents, evidently by the lowest EC_{50} value was found in ethyl acetate partition. Another important finding is the scavenging activities of peel portions were better than meat portions in most cases.

Table 1: EC_{50} Values (μg/mL) of six partitions formed by L-L extractions.

Sample		Partitions					
		E	H	C	EA	B	W
Tainung#2	Meat	ND	2360.1	549.4	320.5	573.8	ND
	Peel	86.6	133.0	45.8	14.5	88.4	ND
Darsan	Meat	ND	328.0	144.6	343.6	288.1	ND
	Peel	305.9	932.7	130.2	38.8	678.3	ND
Purpurea-Roxb	Meat	ND	604.5	359.8	112.8	1525.6	ND
	Peel	130.4	236.2	136.6	23.8	67.2	ND
Vitamin C		21.4					

ND: Not Detected, E: Ethanol, H: Hexane, C: Chloroform, EA, Ethyl Acetate, B: n-Butanol, W: Water Phase.

Figure 1 depicted that temperature effect on DPPH free radical scavenging ratio (SR) of *Purpurea-Roxb* peel portion extracted using semi-continuous HPW process. The suitable extraction condition was found at 300 psig, 120 °C, and 7 g. min/mL. The DPPH free radical was captured up to 71 %. Peels might be scorched when the temperature was approaching 140 °C. Extraction time also limited less than 180 minutes, according to the decreasing trend of scavenging ratio. **Figure 2** revealed the effect of four solid loadings on DPPH scavenging ratio in HPW extraction at 120 °C, 300 psig, and 10 mL/min. The 70-gram solid loading presented the best DPPH scavenging ratio. **Figure 3** disclosed that smaller solid loading gave lower yield than other loadings. **Figure 4** proved that the normalized scavenging ratio of two solid loadings (50 grams and 70 grams) is high might contribute to extract less amount of impurities other than DPPH scavenging compounds.

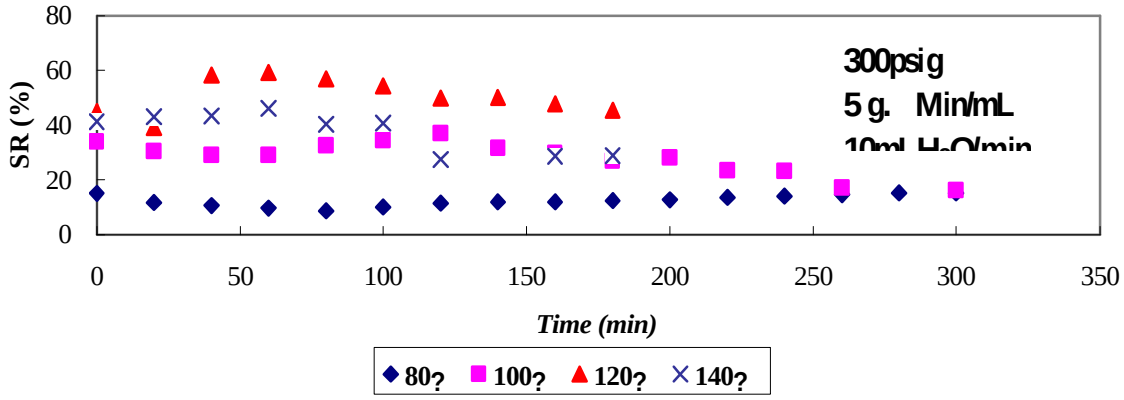

Figure 1: Temperature effect on *Purpurea-Roxb* DPPH scavenging ratio in HPW extraction.

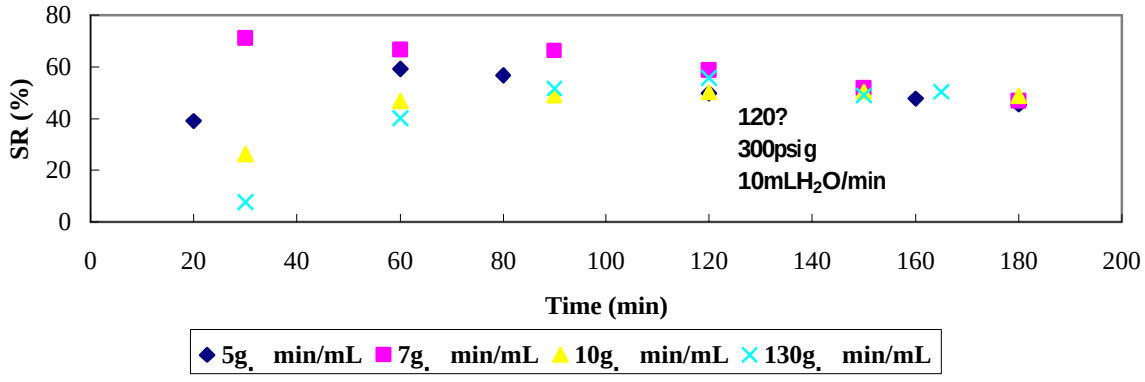

Figure 2: Loading effect on *Purpurea-Roxb* DPPH scavenging ratio in HPW extraction

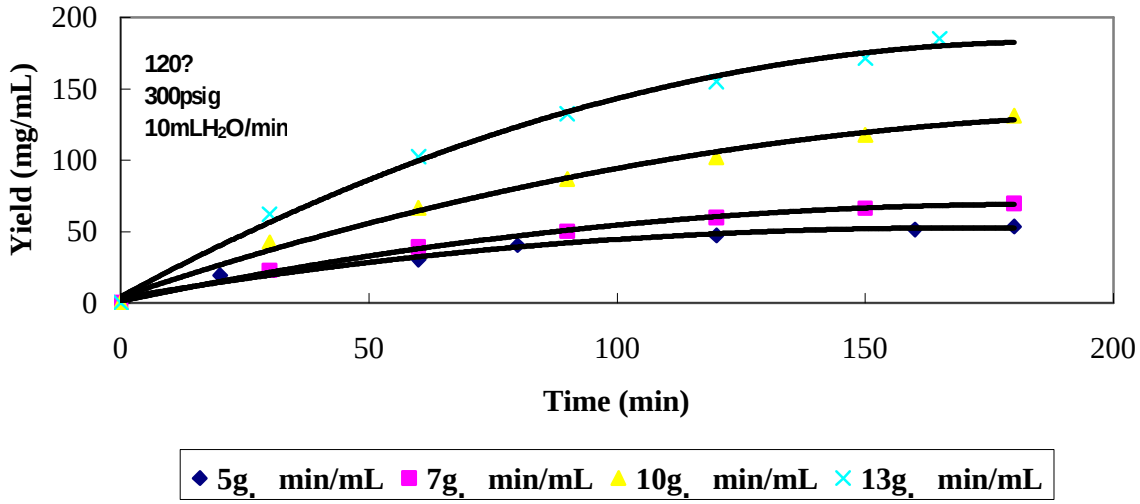

Figure 3: Yields of four solid loadings in HPW extraction.

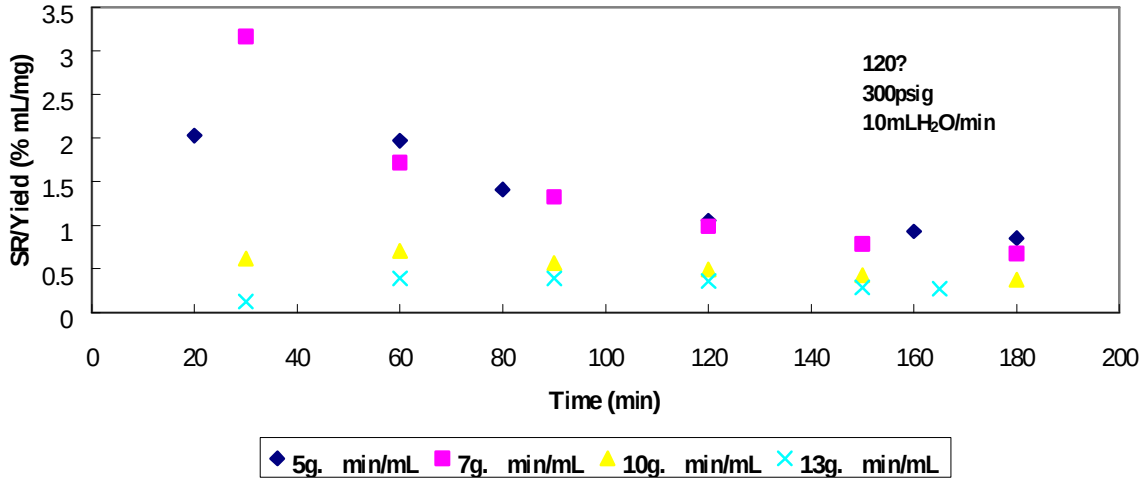

Figure 4: Normalized scavenging ratio of solid loadings in HPW extraction.

CONCLUSIONS

Liquid-liquid solvent extractions and DPPH scavenging tests showed that free radical scavenging compounds extracted from three Taiwan's *Dioscorea alata* have mid to high polarities. Generally speaking, those compounds especially from peel portion, can be extracted more and successfully by using organic solvents. In this study, non-toxic water was also able to extract those antioxidants. Temperature could modulate pressurized water's dielectric constant. The best operating condition of hot pressurized water extraction of DPPH scavenging compounds from yam's peel was found at 120 °C, 300 psig, and 70-gram loading. In other words, 71 % free radicals could be captured by a crude extract using only

water. However, in order to develop a process for the medical usage, obtaining the high purity of active compounds is required. The continuous work is aimed to find out the active compounds followed by doing the purification process.

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