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In vitro Antioxidant Activity of Crude Extract from *Rubus coreanus* M_{IQ}.

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Abstract

Objectives : This research aimed to evaluate the effects of *Rubus coreanus* M_{IQ}.

Methods : These extracts from fresh *R. coreanus* M_{IQ}. were investigated with radical scavenging activity, the content of total phenolics, ferric thiocyanate method and flavonoid contents

Results : These results revealed that the ethyl acetate extract from fresh *R. coreanus* M_{IQ}. possesses remarkable radical scavenging activity. The content of total phenolics of the ethanol extract from dried *R. coreanus* M_{IQ}. (IC_{50} = 155.87 ± 8.8μg/mL) is the highest among all of the samples. The stronger radical-scavenging activity (IC_{50} = 77.72 ± 2.1μg/mL) of ethyl acetate extract from dried *R. coreanus* M_{IQ}. should be related to its phenolic content as measured by gallic acid test.

Conclusion : These results suggest that dried *R. coreanus* M_{IQ}. may act as a potential antioxidant agent.

Key words : *Rubus coreanus* M_{IQ}., radical scavenging activity. phenolics.

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I. Introduction

Fruit characteristics, saccharinity, and antioxidative activity of selected clones of *Rubus coreanus* M_{IQ}. (*R. coreanus* M_{IQ}.) grown in Korea, well-known as edible and medical resources, frequently used and which has an excellent pharmacological activity are examined and analyzed.[1-3] The water extracts from the fruits of *R. coreanus* M_{IQ}. showed antioxidative activity. The unripe fruits of *R. coreanus* M_{IQ}. have been used to remit diabetes mellitus and sexual disinclination. This crude drug contains triterpenoid glycosides such as coreanoside. Nam et. al.[4] reported that treatment with EtOH plus sodium salicylate reduced free radical-scavenging enzyme activities and increased lipid peroxidation, whereas treatment of rats with niga-ichigoside F_1 (NIF_1) and 23-hydroxytormentic acid(23-HTA) attenuated the pathophysiology due to the gastropathic induction. 23-HTA, an aglycone of NIF_1, exhibited more potent activity than NIF_1, thus suggesting that the aglycone may be a more active moiety. Taken together, these research groups suggest that these two species which ameliorated gastropathy have potential as therapies for the long-term treatment of rheumatoid arthritis.[5] Kim et. al.'s[6] results for HBsAg secretion confirmed the inhibitory effects of the four plant extracts on the levels of HBV and DNA released from HepG2 2.215 cells. At 512μg/mL, significant inhibitory effects were observed for the extracts of *T. chebula*, *S. officinalis* and *R. coreanus* with an inhibition of more than 95%, and the extract R. palmatum with 77.9%. Yoon et. al.[7] reported that quercetin isolated from the fruits of *R. coreanum* showed antioxidative activity. The purpose of the work described here is to examine antioxidant properties of *R. coreanus* M_{IQ}. extracts.

II. Materials and Methods

1. Instrument and reagents

Octadecyl-functionalized silica gel, butylated hydroxyanisole(BHA), α-tocopherol and 1,1-diphenyl-2-picrylhydrazyl(DPPH) were purchased from Sigma(St. Louis, MO, USA). All reagents were of analytical grade. Spectrophotometric measurements were performed with a computer-aided UV-VIS spectrophotometer, Hewlett Packard model HP 8453. ^{1}H-NMR spectrum was recorded on a JEOL FT-500 MHz NMR spectrometer. Tetramethylsilane was used as an internal standard.

2. Preparation of Crude Extracts

5g of each dried and fresh *R. coreanus* M_{IQ}. was extracted using ethanol, ethyl acetate and water. Each plant material was macerated in 150ml of ethanol or ethyl acetate for 24 hours at room temperature by continuous stirring. In all cases, the process was repeated thrice. The extracts were then filtered, using Whatman No 42 and evaporated under reduced pressure at 35°C. The aqueous extracts were prepared by reflux using same amount of sample and solvent. The reflux was done thrice at 80°C ~ 85°C for 4 hrs ;

Table 1. Free radical scanvenging activity(DPPH) and total phenolics content of different concentrations of *R. coreanus* M_{IQ} extracts.

Plant(part)	Plant extract	Weight (g)	Yield (%)	DPPH (IC_{50}, μg/mL)	TPC (IC_{50}, μg/mL)
R. coreanus M_{IQ}(dry)	EDRC	0.96	19.27	115.40 ± 0.4****	155.87 ± 8.8
	EADRC	0.96	19.19	77.72 ± 2.1****	143.37 ± 8.8
	WDRC	2.76	55.27	382.07 ± 7.0**	152.75 ± 2.6
R. coreanus M_{IQ}(fresh)	EFRC	0.8	4.00	370.90 ± 3.8**	130.08 ± 2.3
	EAFRC	0.07	0.35	495.19 ± 6.9**	121.95 ± 1.15
	WFRC	1.75	8.75	167.28 ± 7.8	134.15 ± 3.45

Each value is expressed as mean ± S.D. (n = 3). Significantly different from the control values; **p < 0.01. ****p < 0.0001. Plant extracts ; EDRC : Ethanol extract of dried *R. coreanus* M_{IQ}., EADRC : Ethyl acetate extract of dried *R. coreanus* M_{IQ}., WDRC : Water extract of dried *R. coreanus* M_{IQ}. EFRC : Ethanol extract of fresh *R. coreanus* M_{IQ}., EAFRC : Ethyl acetate extract of fresh *R. coreanus* M_{IQ}., WFRC : Ethanol extract of fresh *R. coreanus* M_{IQ}. TPC : Total phenolic content, BHA : Butylated hydroxy anisole(IC_{50}, μg/mL), α-Toco ; α-Tocopherol (IC_{50}, μg/mL).

suction filtered using Whatman No 42 and vacuum evaporated at 40°C. The samples were then transferred into sterile vials and stored in the refrigerator until use(Table 1).

3. DPPH assay

The ability of the *R. coreanus* M_{IQ}. extracts to donate hydrogen atoms or electrons was measured from the bleaching of purple coloured methanol solution of DPPH.7 Briefly, 0.5mL of methanolic solution of 0.24mM DPPH was mixed with 0.5mL of *R. coreanus* M_{IQ}. extracts (in methanol) at various concentrations(50~600μg/mL) and reference compounds. The mixture was vigorously shaken and left to stand for 30 min in dark place. The absorbance was then measured at 517 nm against a blank. Radical scavenging activity was expressed as the percentage of DPPH elimination after 30 minutes and calculated as follow.[8-9]

Scavenging ability(%) = $[A_0 - A_1 / A] \times 100$, where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the test compound. BHA and α-tocopherol were used as positive controls. Moreover, to understand its kinetic behavior, the decrease in absorbance of *R. coreanus* M_{IQ}. extracts and reference standards was evaluated at 517 nm for every minute until 90 minutes.

4. Total phenolic content

The total phenolic content was determined following the Folin-Ciocalteu method.[10] The reaction mixture containing 20mL of sample, 1.58mL water and 100mL of the Folin-Ciocalteu reagent was mixed thoroughly. After one minute, 300mL of 20% sodium carbonate solution was added and shaken well to mix. After 2hrs incubation at room temperature the absorbance was measured at 765nm against the blank(solution contains all the reaction reagents except the sample). Gallic acid(500mL) was used for calibration of a

standard curve. Total phenolic content was determined as gallic acid equivalents(GAE) and values were expressed as mg of acid/g of plant material(in GAE).

5. Ferric thiocyanate method

The FTC method reported by Osawa and Namaki was performed.[8] Sample(4mg/mL) in 99.5% ethanol were mixed with 2.5% linoleic acid in 99.5% ethanol(4.1mL), 0.05M phosphate buffer(pH=7.0) and distiled water(3.9mL) and kept in screw cap containers under dark conditions at 40℃. To 0.1mL of this solution was added to 9.7mL of 75% ethanol and 0.1mL of 30% ammomium thiocyanate. After 3min, 0.1mL of 0.02M ferric chloride in 3.5% hydrochloric acid was added to the reaction mixture, the absorbance of reaction colour was measured at 500nm in spectrophotometer, for every two days. The control and standard were subjected to the same procedure except for the control, where there wae no addition of sample and for the standard 4mg of sample were with 4mg of BHA and α-tocopherol.[11-12]

6. Flavonoid contents

The total flavonoid content was determined according as the aluminum chloride colorimetric method. Briefly, aliquots of 0.1g of samples were, respectively, dissolved in 1mL deionized water. This solution(0.5ml) was mixed with 1.5mL of 95% alcohol, 0.1mL of 10% aluminum chloride hexahydrate($AlCl_3$), 0.1mL of 1M potassium acetate(CH_3COOK), and 2.8mL of deionized water. After incubation at room temperature for 40min, the reaction mixture absorbance was measured at 415nm against a deionized water blank on a spectrophotometer. Quercetin was chosen as a standard. Using a seven point standard curve(0~50mg/L), the total flavonoid contents in samples were determined in triplicate. The data were expressed as milligram QE(quercetin equivalents)/g lyophilized powder. The data were then converted into milligram QE(quercetin equivalents)/g/100g fresh matter from fruit or vegetables based on the moisture content of lyophilized powder and fresh fruit and vegetable materials.[13]

7. Statistical analysis

Microsoft Excel was used to compute mean and standard deviation. Differences among all sample means were determined by analysis of variance(ANOVA) using Origin, version 5 (Micro cal Software, Inc, 1991-97) and were considered significant at $p<0.05$.

III. Results and Discussion

Radical scavenging activities are important due to the deleterious role of free radicals in foods and in biological systems. DPPH assay evaluates the ability of antioxidants to scavenge free radicals. Free radical scavenging capacities of the extracts, measured by DPPH assay, are shown in Table 1. The free radical scavenging activity of ethyl acetate extract from dried R. coreanus M_{IQ}(IC_{50} = 77.72 ± 2.1μg/mL) was superior to all other extracts. However, the stronger activity of

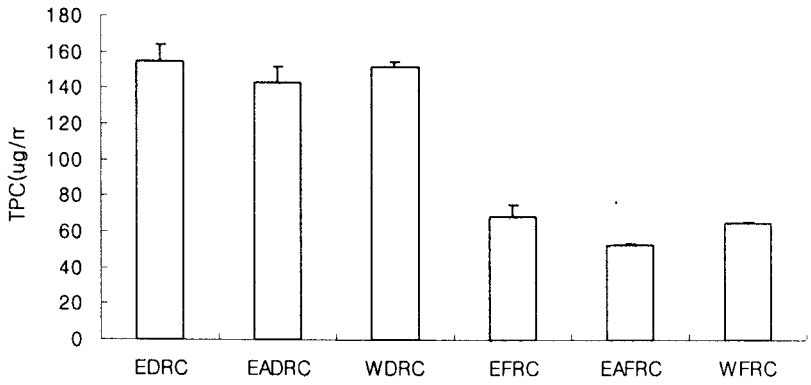

Fig. 1. Total phenolic content of *R. coreanus* MIQ. extracts. Plant extracts; EDRC : Ethanol extract of dried *R. coreanus* MIQ., EADRC : Ethyl acetate extract of dried *R. coreanus* MIQ., WDRC : Water extract of dried *R. coreanus* MIQ. EFRC : Ethanol extract of fresh *R. coreanus* MIQ., EAFRC : Ethyl acetate extract of fresh *R. coreanus* MIQ., WFRC : Water extract of fresh *R. coreanus* MIQ.

ethyl acetate extract from dried R. coreanus MIQ should be related to its less phenolic content(IC_{50} = 143.37 ± 8.8μg/mL) as measured by gallic acid test(Fig. 1).[7] Generally, the free radical scavenging activity of the dried *R. coreanus* MIQ extracts was more antioxidant activity than the fresh *R. coreanus* MIQ extracts. The scavenging effects of the crude extracts from R. coreanus MIQ and antioxidant standard such as butylated hydroxy anisole and α-tocopherol on the DPPH radical decreasee in the order; α-Tocopherol > BHA > EADRC > EDRC > WFRC > EFRC > WDRC > EAFRC, respectively. Free radical scavenging activity of these samples also increased with concentration dependent manners (Table 1).

From the results shown in Table 1, the content of total phenolics of the ethanol extract of dried R. coreanus MIQ.(IC_{50} = 155.87 ± 8.8μg/mL) is the highest among all of the samples. The stronger radical-scavenging activity (IC_{50} = 77.72 ± 2.1μg/mL) of ethyl acetate extract from dried *R. coreanus* MIQ. should be related to its higher phenolic content as measured by gallic acid test (Fig. 1).[5] Based on the absorbance values of the various extract solutions, reacting with Folin-Ciocalteu reagent and compared with the standard solution of gallic acid equivalents, as described above, the total phenolics content was highest in EDRC(IC_{50} = 155.87 ± 8.8μg/mL), followed by WDRC(IC_{50} = 152.75 ± 2.6μg/mL), EADRC(IC_{50} = 143.37 ± 8.8μg/mL), WFRC(IC_{50} = 134.15 ± 3.45μg/mL), EFRC (IC_{50} = 130.08 ± 2.3μg/mL), and EAFRC(IC_{50} = 121.95 ± 1.15μg/mL). The lowest amount of total phenolics was shown in ethyl acetate soluble extract of fresh R. coreanus MIQ.[7]

In our results, the extract from *R. coreanus* MIQ. that showed strong antioxidant activity and scavenging effect on DPPH could be correlated with their contents of total phenolic

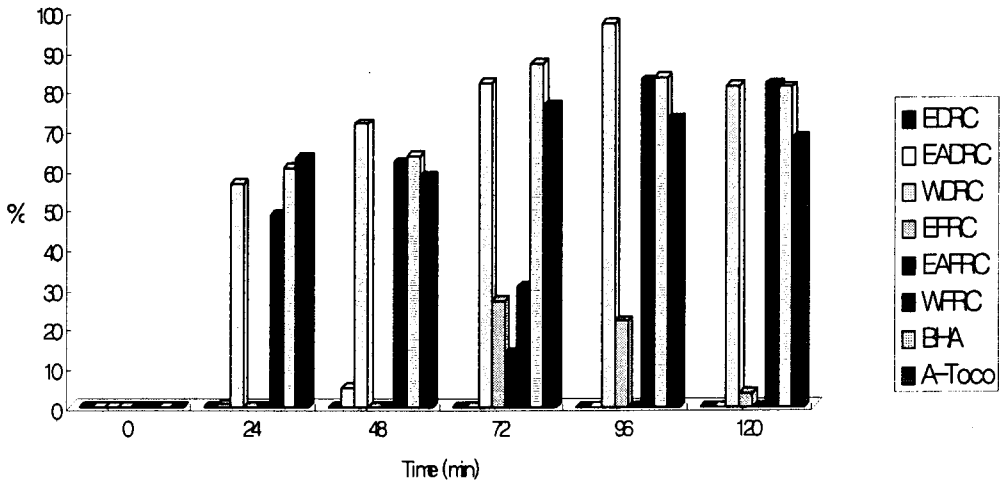

Fig. 2. Total antioxidant activities of 4mg/mL concentrations of *R. coreanus* M_{IQ}. extracts, BHA and α-tocopherol were determined by ferric thiocyanate method. Plant extracts; EDRC: Ethanol extract of dried *R. coreanus* M_{IQ}., EADRC: Ethyl acetate extract of dried *R. coreanus* M_{IQ}., WDRC: Water extract of dried *R. coreanus* M_{IQ}. EFRC: Ethanol extract of fresh *R. coreanus* M_{IQ}., EAFRC: Ethanol extract of fresh *R. coreanus* M_{IQ}., WFRC: Ethanol extract of fresh *R. coreanus* M_{IQ}. BHA : Butylated hydroxy anisole, A-Toco : α-Tocopherol.

compounds. The molecular mechanism of radical scavenging activity of ethanol extract from dried *R. coreanus* M_{IQ}. could be attributed to the presence of polyphenolic compouds. It has already been shown that polyphenolic compounds were responsible for radical scavenging activity in Rosaceae family due to ease of their hydrogen atom donation to active free radical.[12-13] This may be due to the synergistic effect of other phenolic compounds present in the crude extract, which supplement the radical scavenging ability. Both ethanol extract and ethyl acetate extract showed a potential radical scavenging activity.

The extract from *R. coreanus* M_{IQ}. and standard compounds exhibited effective total antioxidant activity. These results are represented in Fig. 2. The inhibition of peroxidation in linoleic acid system by 4mg/mL concentration of the extract from *R. coreanus* M_{IQ}. was found to be 55%. However, 4mg/mL concentration of EDRC, EADRC, WDRC, EFRC, EAFRC, WFRC, BHA, α-tocopherol had % inhibition of peroxidation on linoleic acid emulsion system, respectively. Total antioxidant activity of 4mg/mL concentration of the extract from *R. coreanus* M_{IQ}. and standard compounds exhibited the following order: WDRC > BHA > WFRC > α-tocopherol > EFRC > for days. However, EDRC, EADRC, and EAFRC did not exhibited.

Total antioxidant activity of the extract from *R. coreanus* M_{IQ}. was determined by ferric thiocyanate method in linoleic acid emulsion

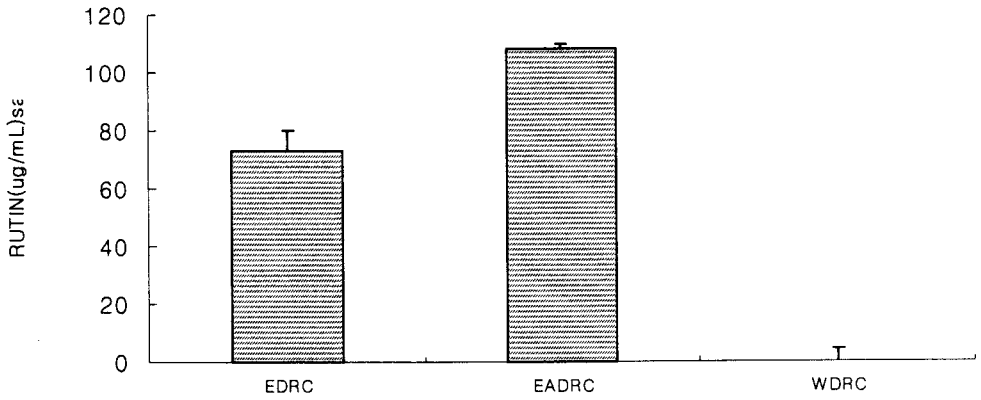

Fig. 3. Flavonoid content of *R. coreanus* Miq. extracts. Plant extracts ; EDRC : Ethanol extract of dried *R. coreanus* Miq., EADRC : Ethyl acetate extract of dried *R. coreanus* Miq., WDRC : Water extract of dried *R. coreanus* Miq.

system. As shown in Fig. 3, EDRC, EADRC, WDRC, EFRC, EAFRC, WFRC, and standard compounds was determined by the ferric thiocyanate method in linoleic acid emulsion system. As shown in Fig. 3, WDRC had effective total antioxidant activity than standards antioxidant compounds such as BHA and α-tocopherol, at same concentration.[14]

From analysis, we can deduce that EADRC, and EDRC are rich in flavonoids exception of WDRC. We outline that the amount of flavonoids in the fruit plant varies from 108 ± 1.5 to 73 ± 6.7μg/mg rutin equivalent of the crude extract. However, we can state here that an extraction method must be remove nonphenolic substances such as sugars, proteins and pigments which may interfere during the total phenolic evaluation. Upon such extraction, we have obtained results which show a highly total amount of the phenolics significantly. We also mention here that an increase of the phenolic metabolism in this plant may be related to the hard climate conditions(hot temperatures, high solar exposure, dryness, short growing season.[15]

We evaluated the ethyl acetate extract from fresh R. coreanus MIQ. by nuclear magnetic resonance spectrometry(JEOL FT-500MHz, Janpan). The ethyl acetate extract was analysed by ^{1}H-NMR spectroscopy of $CDCl_3$ solubles. It gave the main biological compounds, which the signals were clearly visible in the ^{1}H-NMR spectrum. Perhaps, the obvious chemical shifts were between weak 7.5ppm and 7.7ppm indicative of phenyl group, olefinic groups between 4.1ppm and 5.4ppm, alkyl groups between 0.7ppm and 1.8ppm in aromatic compounds.[16]

IV. Conclusion

In summary, the present results revealed that the ethyl acetate extract from fresh *R. coreanus* Miq. possesses remarkable radical sca-

venging activity. The content of total phenolics of the ethanol extract from dried *R. coreanus* M_{IQ}.(IC_{50} = 155.87 ± 8.8μg/mL) is the highest among all of the samples. The stronger radical-scavenging activity(IC_{50} = 77.72 ± 2.1μg/mL) of ethyl acetate extract from dried *R. coreanus* M_{IQ}. should be related to its phenolic content as measured by gallic acid test. This ethyl acetate extract was concentration dependent manners. It might be effective against diseases caused by over production of radicals. We hope that these results obtained will provide a starting point for the further investigations specially to evaluate its in vivo potential in animal models and the different antioxidant mechanisms.

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