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Antioxidant Activity and Macropharge Cell Proliferation of KoreanBulgeun(Reddish) Mistletoe (*ViscumAlbum* For. *Rubroautiacum*Ohwi)

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Abstract: *This study was conducted to investigate the food and medicinal functionality of Viscum album var. for. rubroaurantiaum hemiparasitic plant. Morphological characteristics, total polyphenol, total flavonoid, DPPH scavenging activity, macropharge cell proliferation, and nitric oxide production were analysedof four different plant parts such as leaf, node, bract, and fruit. Among the diferent plant parts investigated, content of total polyphenol ranged 21.1-24.6 mg/g TAE while total flavonoid ranged 5.5-10.7 mg/g RUE. The highest content of TP and TF were found in leaf, showing 24.6 mg/g TAE and 10.7 mg/g RUE, respectively. The highest DPPH free radical scavenging activity was observed in bract with 87.1%. The extract of each plant part obtained showed different cell proliferation values ranging from 98.5% (node) to 125.6% (fruit), both at the concentration of 500 µg/mL. Nitric oxide content in four partsliesbetween 5.5-26.5 µmol/L was all lower than LPS showing 46.3 µmol/L.The results of this study show that Viscum album var. for. rubroaurantiaum has antioxidant and immunomodulating activity and thus potentially can be usedas a functional food and medicine.*

Keywords: *Antioxidant, cell proliferation, mistletoe, macropharge,nitric oxide,total phenol,Viscum album var. for. rubroaurantiaum*

I. Introduction

Mistletoes (*Viscum album*) are highly specialized angiosperms of the family Loranthaceae, which are well known as broad host range hemi-parasites of a variety of different gymnosperms and angiosperms[1]. Genus *Viscum*possesses about 70-100 species of mistletoe, native from Asia, Africa, Europe, and Australia[2]. They occur ubiquitously in the temperature zone and in arid regions, but the large majority of mistletoe taxa is found in the tropics. There are three genus and 6 species of mistletoe in Korea; [*Viscum album* var. *coloratum* (Kom.) Ohwi], *Viscum album* var. for. *rubroaurantiaum* Ohwi, *Loranthus tanakae* Fr. et, *Loranthus yadoriki* Sieb, *Loranthus parasiticus* Merr., and *Pseudixus japonicus* Hayata[3]

Mistletoe is of great economic importance due to its major use in the medical treatment and management of many diseases for many year, both in traditional and complementary medicine in Korea. A number of biological effects such as anticancer, antimycobacterial, antiviral, apoptosis-inducing and immunomodulatory activities have been reported for mistietoe[4,5].Previousresearch reavled that mistletoe has higher antioxidant activity that has been using for treatment of many diseases.[6,7,8].However, still there is no research reported on Korean mistletoe, *Bulguen* mistletoe(*V. album* var. for. *rubroaurantiaum* Ohwi) consideraing its antioxidant activity Macropharges are known to be closely related to the non-specific immune reponses and play an important role in releasing cytokines and chemokines, especially nitric oxide(NO). The released NO from macropharge is a highly reactive free radical and an important molecule in the control of immune system[9].Therefore the aim of this study was to determine total polyphenol content, antioxidant activities, and macropharge cell proliferation and NO releasing capacity of Korean mistletoe (*V. album* var. for. *rubroaurantiaum* Ohwi)extracts from each organ such as leaf, node, bract, and fruit for the purpose of developing functional food and medicinalvalue.

II. Materials And Methods

Plant materials

Bulguen(reddish) mistletoe(*V. album* var. for. *rubroaurantiaum*) was collected from a host treeMongolian oak tree (*Quercus mongolica* Fischer). Organs such as leaf, node, fruit, bract, and seed were separated from each other and investigated for morphological characteristics. Size including length, width, and thickness and fresh weight of each organ were measured and then dried for the analyses of total content in phenol and flavonoid.

Estimation of total polyphenol

Total phenolic content was estimated by the Folin-Ciocalteu assay. In brief, a sample aliquot of 0.2 ml of extract (1 mg/mL) was added to the test tube containing 0.2 ml of phenol reagent (1 M). The volume was increased by adding 1.8 mL of deionized water and the solution was vortexed and left for 3 min for reaction. Furthermore, 0.4 mL of Na_2CO_3 (10% in water, V/V) was added and the final volume (4mL) was adjusted by adding 1.4mL of deionized water. The absorbance was measured at 725 nm after incubation for 1 hour at room temperature. The TPC was calculated from a calibration curve (R^2=0.999) using tannic acid as standard and expressed as mg of tannic acid equivalent (TAE)/g (DW).

Estimation of total flavonoid content

The total flavonoid content was determined according to Sharma *et al.*[10]with minor modifications. In brief, an aliquot of 0.5 mL of sample (1 mg/mL) was mixed with 0.1 mL of 10% aluminum nitrate and 0.1 mL of potassium acetate (1 M). In the mixture, 3.3 mL of 70% ethanol was added to make the total volume 4 mL. The mixture was vortexed and the absorbance was measured after 40 min at 415 nm in spectrophotometer and calculated. Rutin was used as a standard and the values of TF content were expressed in rutin equivalent (RUE) mg/g (DW).

Estimation of DPPH free radical scavenging activity

The antioxidant activity of mistletoe extracts was determined on the basis of the scavenging activity of the stable 1,1-diphenyl-2- picryhydrazyl (DPPH) free radical according to the method described by Ghimeray *et al.*[11] with slight modifications. Briefly, 1ml of the extract at different concentrations (0.25, 0.5 and 1 mg/mL) was added to 3 mL (100% of methanol) of DPPH. The mixtures were shaken vigorously and left to stand at room temperature in the dark for 30 minutes. The absorbance was measured at 517 nm in a spectrophotometer (Model: UV-1800 240V, Shimadzu Corporation, Kyoto, JAPAN). All determinations were performed in triplicate and the scavenging activity of the extracts was calculated against a blank:Radical scavenging activity (%) = (A0 –A1)/A0 x 100, Where, A0 and A1 were the absorbance of the control and the test sample, respectively.

Estimation of macrophage cell proliferation and nitric oxide (NO)

The macrophage cell line (RAW264.7) in RPMI–1640 medium containing 10% FBS was plated in 96 well–plate (1×10^5cells/well; obtained from ATCC) and incubated with the addition of the mistletoe extracts (60, 125, 250, and 500 µg/mL) in triplicate. After a 24 hour incubation of cells in a humidified atmosphere containing 5% CO_2 at 37°C, 20µl of the WST–1 solution was added to the well, and the solution was further incubated for 4 hours at 37°C. The optical density was measured at 450 nm using a microplate reader (EL–800, BioTek Instruments, Winooski, VT, USA). The absorbance was translated into macrophage proliferation ratio (%) = At/Ac×100, where At and Ac are the absorbance of the test group and control group, respectively. The nitric oxide (NO) production in RAW264.7 cell culture supernatant (1×10^5cells/well), incubated in the mistletoe extracts at different concentrations (60, 125, 250, and 500 µg/mL) and LPS (1 µg/mL) at 37°C for 24 houras previously described [12]. The NO production of the cells was calculated with reference to a standard curve obtained with $NaNO_2$(1–200µM in culture medium).

Statistical analysis

All data were expressed as the mean value ± standard deviation (SD) of each experimental group (n=3). The Differences in the mean values among samples were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple tests using SPSS 16.0 (SPSS Inc., USA). Statistical significance was considered at $P< 0.05$.

III. Results And Discussion

Plant morphology

Morphological characteristics of organs in *Viscum album* for. *rubroautiacum* were shown in Table 1. Length of leaf and node were respectively 50.6 mm and 46.8 mm. Diameter of fruit was 7.7 mm and it weighed 0.36 g in fresh weight basis. Length and fresh weight of bract were respectively 3.2 mm and 0.2 g. Seed was greenish and sized with 5.3 cm(length) x 3.6 cm(width) x 1.7 mm(thickness). Leaf size in this study was smaller than that (ranging 52.9 - 75.6 mm) of a few previous studies of mistletoes, of which host tree were different [13,14].

Table 1. Morphological charateristics of leaf, node, fruit, bract, and seed in *V. album* var. for. *rubroaurantiaum*

Plant part	Characteristics	
Leaf	Length x width x thickness (mm)	50.59 x 12.71 x 1.02
	Fresh weight (g)	0.17±0.04
Node	Length x thickness (mm)	46.76 x 2.50
	Fresh weight (g)	0.14±0.04
Fruit	Type	Berries
	Color	Orange
	Diameter (mm)	7.68±0.59
	Fresh weight (g)	0.36±0.05
Bract	Length (mm)	3.16±0.30
	Fresh weight (g)	0.21±0.04
Seed	Color	Green
	Length x width x thckness (mm)	5.30 x 3.61 x 1.71

Fig. 1. Photographs of leaf and node, fruit, and bract (clockwise) in.*V. album* var. for. *Rubroaurantiaum*

Total polyphenol and total flavonoid content

Phenolic compounds, such as quercetin, rutin, narigin, catechins, caffeic acid, gallic acid, and chlorogenic acid, are very important plant due to their antioxidant activities[15]. Significant differences in contents of total polyphenol(TP) and total flavonoid(TF) were found among different organs of the mistletoe plant, even though there are not much different (Fig. 2 and 3). Among the organs investigated, content in total polyphenol ranged 21.1-24.6 mg/g TAE while that of total flavonoid ranged 5.5-10.7 mg/g RUE. The highest content in TP and TF were found in leaf, showing 24.6 mg/g TAE and 10.7 mg/g RUE, respectively. On the other hand, node gave the lowest contents (21.1 mg/g TAE) in TP and fruit showed the lowest contents (5.5 mg/g RUE) in TF. The contents in TF of leaf extract was two times higher compared to fruit extract.

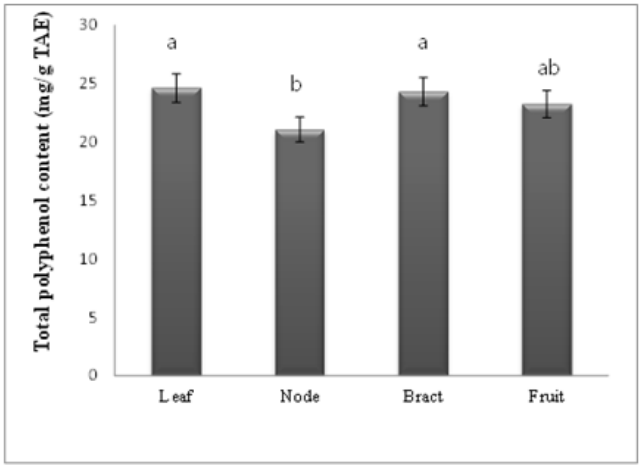

Fig. 2. Total polyphenol content (mg/g TAE) of *V. album* var. for. *rubroaurantiaum*

Flavonoids are ubiquitous in plants and form a group of low molecular weight polyphenolic compounds. Numerous bioactive compounds, mainly phenolic acid and flavonoids, are found in cereal/whole grains[16]However, content in phenolics of sprouts, vegetables and fruits can be affected by genetic and environmental factors like cultivar, illumination, and temperature [17]. Ademiluyi and Oboh [18] found that TP content was 182 mg/100g in Cocoa tree mistletoe and 160 mg/100g in Cashew tree mistletoe. Simirgiotis *et al.* [19] reported 37.3 µg/mL and 26.8 mg/g (quercetin equivalent) in TF from leaves of Chilean mistletoe while those from flowers were 24.6 µg/mL and 17.5 mg/g (quercetin equivalent) in TF. Ju *et al.* [20] reported total polyphenol of 24.7 mg/g and 15.1 mg/g, respectively from 50% and 100% ethanol extract while Lee *et al.*[6] demonstrated that TP content was 90.3-109.7 mg/g in the concentration of 25 mL/g. Lee *et al.* [21] found that water extract from *Viscum album* showed the highest TP with 148.2 mg/g, compared to 50% and 100% ethanol extract.

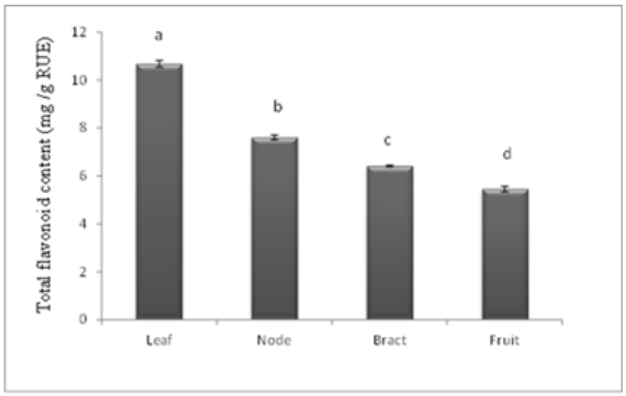

Fig. 3. Total flavonoid content (mg/g RUE) of *V. album* var. for. *Rubroaurantiaum*

DPPH Free radical scavenging activity

DPPH is a stable free radical. Researchers have used this compound to evaluate the efficiency of antioxidants and flavonoids. The DPPH radical has been widely used to measure the free radical scavenging activity of various natural products and is accepted as a model compound of free radical originating from lipids [22].

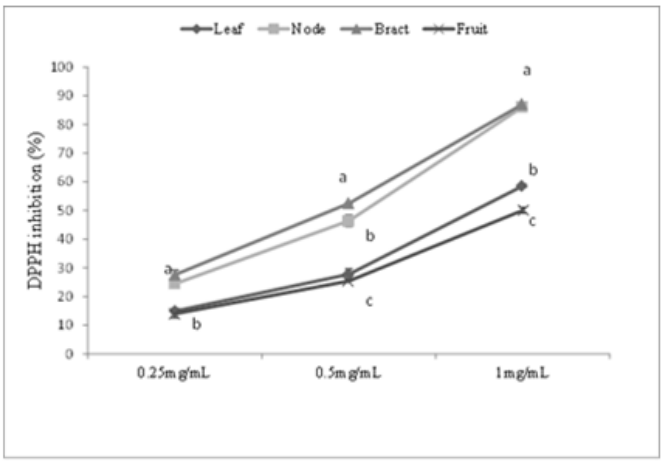

Figure. 4. DPPH free radical scavenging activity (%) of *V. album* var. for. *Rubroaurantiaum*

In the present study, DPPH free radical scavenging activity of mistletoe extracts was determined at concentrations of 0.25 mg/mL, 0.5 mg/mL, and 1 mg/mL. The highest DPPH free radical scavenging activity was observed in bract with 87.1%, followed by node and leaf with 86.2% and 58.6%, respectively at the concentration of 1 mg/mL (Fig. 4). Many researchers have previously made an effort to elucidate the antioxidant activity of mistletoe, *Viscum abum*. by scavenging DPPH free radical of which results were not so much different as this study. Onay-Ucar *et al.* [23] reported that *Visum album* growing on lime tree exhibited the highest activity of DPPH inhibition with 95.1%. Ju *et al.* [20] demonstrated that DPPH was 80% in 50% ethanol extract but decreased to 50% in water extract and 100% ethanol extract while Lee *et al*· [6] showed 60% ethanol extract resulted in 68.9% of DPPH in the concentration 25 mL/g from mistletoe extracts. Simirgiotis *et al.*[19] showed respectively 13.4 µg/mL and 23.4 µg/mL in DPPH of leaves and flowers in Chiean mistletoe, indicating significantly higher DPPH scavenging activity than those of gallic acid (1.5 µg/mL) and quercetin (9.7 µg/mL).

Macrophage cell proliferation and nitric oxide inhibition

Various concentrations of mistletoe extracts (60, 125, 250, and 500 µg/mL) from the different organs were used to quantify cell proliferation. Overally, mistletoe extracts possessed higher cell proliferation as compared to the positive control(medium). Except for leaf and node, cell proliferation of bract and fruit extracts in this study was increased up to the concentration of 500 µg/mL. The extract of each organ obtained showed different cell proliferation values ranging from 98.5% in minimum (node) to 125.6% in maximum (fruit), both at the concentration of 500 µg/mL. The second highest cell proliferation was observed in bract extracts (124.9%) at the concentration of 500 µg/mL. All of four organs showed in average the highest cell proliferation values at the concentrations of 250 and 500 µg/mL, which were 12% higher than the control.

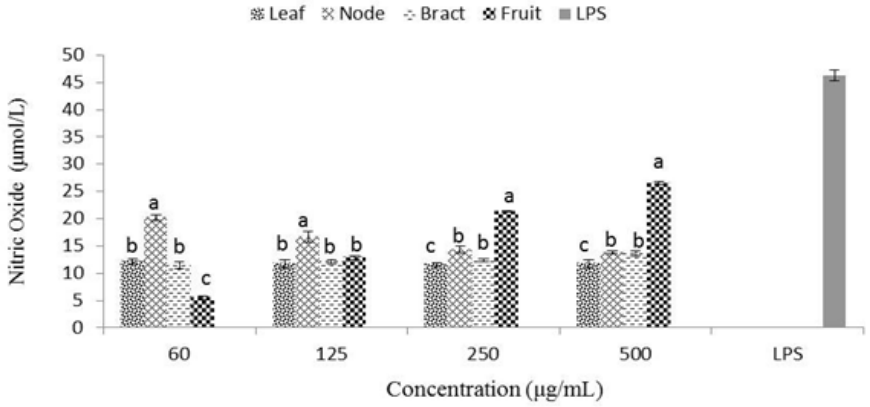

Fig. 5. Macrophage cell proliferation of *V. album* var. for. *rubroaurantiaum* (MD means medium)

The mistletoe extracts obtained from different organs was assayed for Nitric oxide (NO) production. Various concentrations of the extracts (62.50, 125, 250, and 500 µg/mL) were prepared and NO production was assayed. Nitric oxide content in four organs ranging 5.5-26.5 µmol/L was all lower than LPS showing 46.3 µmol/L. The lowest NO production of all extracts obtained from four different organs was observed in fruit (5.5 µmol/L) at a concentration of 60 µg/mL of sample extract followed by bract (11.5 µmol/L) at a concentration of 60 µg/mL and node (11.6 µmol/L) at a concentration of 250 µg/mL, respectively. This study revealed that fruit extract of VAR has a high potential inhibitory activity of NO at the lowest concentration of 60 µg/mL.

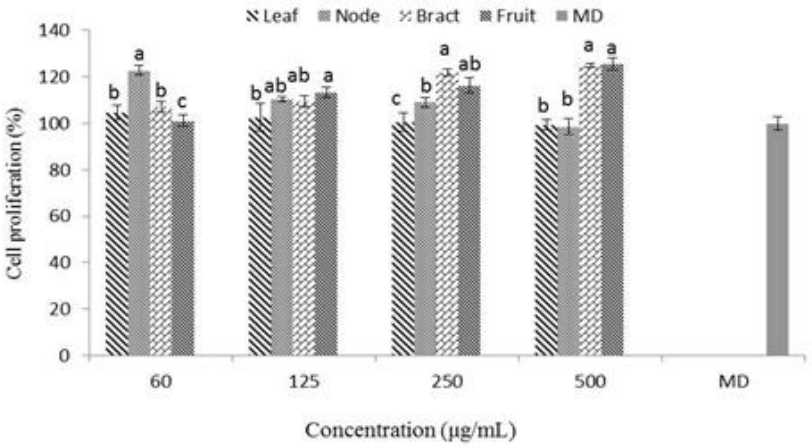

Fig. 6. Nitric oxide of *V. album* var. for. *Rubroaurantiaum*

The inhibitory activity of NO production in lipopolysaccaride (LPS) activated RAW 264.7 cells was reported in several Korean medicinal plants [24,25,26]. Conclusively, our finding confirms the potential usefulness and effectiveness of Korean reddish mistletoe, *Viscum album* for. *rubroautiacum* as a materials for functional food and medicine, even in any small parts of plant organ, by determining antioxidant and immunomodulating activities

Acknowledgments

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