Asian Journal of Pharmaceutical and Clinical Research
INNOVARE ACADEMIC SCIENCES Knowledge to Innovation
Vol 10, Issue 12, 2017
Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF *CAMELLIA* MISTLETOE (*KORTHALSELLA JAPONICA*) EXTRACTS

MIN YOUNG KIM*

Department of ???, Toxicology Laboratory, Faculty of Biotechnology (Biomaterials), College of Applied Life Science, SARI, Jeju National University, Jeju, Republic of Korea. Email: jeffmkim@jejunu.ac.kr

Received: 31 August 2017, Revised and Accepted: 06 October 2017

ABSTRACT

Objective: This study was aimed to analyze the methanol or ethanol extract of *Camellia* mistletoe (*Korthalsella japonica*) growing in Jeju Island, Korea, for their composition of phenolics, flavonoids, and their antioxidant activity.

Methods: Total flavonoid, total carotenoid and L-ascorbic acid contents were determined by using colorimetric assays, and the antioxidant attributes of the methanol and *Camellia* mistletoe extracts were assayed using *in vitro* models, including the following: Free radical (1,1-diphenyl-2-picrylhydrazyl, $O_2^{-\bullet}$ and $NO^{\bullet}$)scavenging, reducing power, and chelating ferrous ions.

Results: Total flavonoid, total carotenoid and L-ascorbic acid contents were 20.3 and 14.9 mg RE/100 g, 4.4 and 1.7 mg/100 g, and 0.4 and 1.4 mg AA/100 g in the methanol and ethanol extracts of *Camellia* mistletoe, respectively. Based on IC_{50} values, both extracts were also found to possess potent antioxidant activities.

Conclusions: *Camellia* mistletoe extracts could be the good antioxidant source and holds promise as a natural ingredient in functional food and pharmaceutical supplement.

Keywords: *Camellia* mistletoe, Total flavonoid content, Total carotenoid content, Ascorbic acid content, Antioxidant activity.

© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4. 0/) DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i12.22342

INTRODUCTION

Mistletoes (families Loranthaceae and Viscaceae) are small semi-parasitic evergreen shrubs that grow on a variety of trees and are particularly noticeable on leafless winter trees. Mistletoes have recently been described to be both an agricultural pest and a threatened species in different parts of the world, because they live in an intimate association with their hosts, derive nutrition from the host and share a life-long association with a single individual host. Mistletoes are also of great economic importance due to their major use in the medical treatment and management of many diseases for many years. They are used as traditional and complementary medicine in treatment for cough, diabetes, hypertension, cancer, diuretic, smallpox, ulcer, skin infection, and after childbirth [1-3]. It has been suggested that pharmacologically active compounds may pass from a variety of host trees to the parasitic mistletoe plants [4].

Mistletoes are native to Asia, Africa, Europe, and Australia, and 70-100 species have been recorded [5]. There are three genera and six species of mistletoes in Korea [6]. *Camellia* mistletoe, *Korthalsella japonica* (family Santalaceae), is one of the species which is distributed in Jeju Island, Korea. Although previous research revealed that mistletoe has a higher antioxidant activity that has been using for treatment of many diseases, most investigative work has been done on the European mistletoe, *Viscum album* L. [7]. Therefore, the present study was aimed to analyze the bioactive contents of total flavonoids, total carotenoids and L-ascorbic acid, and investigate *in vitro* antioxidant activities of methanol and ethanol extracts of *Camellia* mistletoe (*K. japonica*) through radical (1,1-diphenyl-2-picrylhydrazyl [DPPH], $O_2^{-\bullet}$ and $NO^{\bullet)}$ scavenging, ferrous ion chelating and ferric reducing power. We also extended our previous work [8]by harvesting plants at a different time and optimizing sample preparation procedures.

METHODS

Chemicals and reagents

Rutin, aluminum chloride, metaphosphoric acid, L-ascorbic acid, sodium nitrite, DPPH, sodium nitroprusside, Griess reagent, ferric chloride, potassium ferricyanide, and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich (MO, USA). All ingredients used in this study were of analytical grade.

Plant material and extraction procedure

Camellia mistletoe (*K. japonica*) samples were collected during May 2015 from Camellia Hill located in Andeok-myeon, Seogwipo-si, Jeju-do Province, South Korea, and the voucher specimens (KHUP-0803) have been deposited in the herbarium of College of Applied Life Science, Jeju National University. Samples were prepared using a previously described protocol [9]. Briefly, the plant sample was rinsed, weighed and then ground into a fine powder using a blender. Portions (20 g) of the powdered sample were extracted with 100 mL of 100% methanol or 70% ethanol for 72 hrs at room temperature with constant shaking. The mixture was then centrifuged at 1000×g for 15 minutes and the supernatant then purified using a Sep-Pak C_{18} cartridge and a 0.45 µm membrane filter (Waters, Milford, MA, US), concentrated using a rotary evaporator (Buchi Rotavapor R-200, New Castle, DE, US), freeze-dried and stored at −20°C in storage vials for experimental use.

Total flavonoid content (TFC)

TFC of the extracts was determined by the aluminum chloride colorimetric method [10]. In brief, 15 µL of each extract in methanol was mixed with 4.5 µL of 5% $NaNO_2$, 60 µL of distilled water, and 4.5 µL of 10% $AlCl_3$. After incubation for 6 min, 60 µL of NaOH solution (4%) was added to the mixture and made up to a final volume of 150 µL with distilled water. The mixture was allowed to stand for 15 minutes, and absorbance was measured at 510 nm. TFC was calculated from a

calibration curve, and the result was expressed as mg rutin equivalent (RE) per 100 g dry weight (mg RE/100 g).

Total carotenoid content

An aliquot of the extracts was used for quantification of total carotenoid content (TCC) using a Spectra MR microplate reader (Dynex Technologies, Inc., Chantilly, VA, US). TCC was calculated by measuring the absorbance at 470, 653, and 666 nm according to the equations reported previously and expressed as mg/100 g [11]. All operations were carried out on ice under dim light to prevent photodegradation, isomerizations and structural changes of the carotenoids.

Ascorbic acid content

Ascorbic acid content of the extracts was determined following the procedures as described earlier [12]with some modifications. The extract (0.15 g) was treated with 10 mL of 1% metaphosphoric acid (pH 1.86) in a rotary mixer at 200 rpm for 45 minutes in the dark. After centrifuging at 1600×g and 4°C for 15 minutes, the supernatant was collected. A portion of supernatant (25 μL) was mixed with 225 μL of 2,6-dichloroindophenol (0.3 mg/mL), and the absorbance was measured at 515 nm within 15 seconds. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid and was expressed as mg ascorbic acid per 100 g dry matter (mg AA/100 g).

Scavenging of free radicals

DPPH, superoxide anion and nitric oxide free radical scavenging activities of extracts were determined as described in our recently published paper [10]. A dose-response curve was plotted to determine the IC_{50} values which are defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests were performed in triplicate.

Ferrous ion chelating

The ferrous ion chelating ability of extracts was determined as described earlier [10]. Briefly, 250 μL of the extract was mixed with 5 μL of 2 mM ferrous chloride ($FeCl_2$). The reaction was initiated by the addition of 10 μL of 5 mM ferrozine and then incubated at 25°C for 10 minutes. The absorbance of the reaction mixtures was measured at 562 nm against blank samples.

Ferric reducing power

The Fe^{3+} reducing power of extracts was determined by the method of Huang *et al*. [13]. The extract (200 μL) at various concentrations was mixed with 200 μL of 0.2 M phosphate buffer (pH 6.6) and an equal volume of potassium ferricyanide [$K_3Fe(CN)_6$](1%, w/v), followed by incubating at 50°C for 20 minutes. The reaction was stopped by adding 200 μL of 10% TCA solution and then centrifuged at 800×g for 10 minutes. 100 μL of the supernatant was mixed with 100 μL of distilled water and 20 μL of 0.1% (w/v) ferric chloride solution and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical analysis

Data were analyzed using SPSS statistical software (SPSS, ver. 12.0; SPSS Inc., Chicago, IL, US). All measurements were carried out in replicate (n=3). A dose-response curve was plotted to determine the IC_{50} values which are defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. Comparisons of all results between methanol and ethanol extracts of *Camellia* mistletoe were made using a non-parametric test (Mann–Whitney U test)with p<0.05.

RESULTS AND DISCUSSION

Phytochemical contents

Phenolic compounds, aromatic secondary plant metabolites, which mainly include flavonoids, carotenoids, and L-ascorbic acid, and exhibit high antioxidant activity, and their consumption has been linked to a decreased risk of developing chronic and degenerative diseases [14]. In this study, the total flavonoid, total carotenoid and ascorbic acid contents, as well as antioxidant activities (radical scavenging, ferrous ion chelating and ferric reducing power) of methanol and ethanol extracts of *Camellia* mistletoe (*K. japonica*) were quantified. TFC of the methanol and ethanol extracts, calculated from the calibration curve (R^2=0.999), were 20.3 and 14.9 RE g/100 g, respectively (Table 1). The methanol extract contained higher total flavonoids than the ethanol extract (p<0.05). Flavonoids, including flavones, flavanols, and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo* [15]. Recently, Vicas *et al*. [16]reported that the TFC of the ethanol extracts of mistletoe *V. album* harvested from six different hosts were 0.05-2.19 mg RE/100 g, which is 6.8-324 folds lower than that of the ethanol extract (14.9 mg RE/100 g) in our study (Table 1). This implies that differences of secondary metabolites in mistletoes are influenced by the kind of host plants and the type of solvents used for extraction.

Oxidative properties of plant extracts were tested by determining the contents of essential cellular antioxidants that are enzymes SOD, POX, and low molecular weight non-enzymatic antioxidants: Carotenoids and ascorbic acid and glutathione [17]. Carotenoids, vegetable dyes present in the chloroplasts and chromophores, play an auxiliary role in the process of photosynthesis [18]. In addition, ascorbic acid, as well as carotenoids also have a protective function against photo-oxidation processes. Carotenoids and ascorbic acid present in the tissues of studied plants may also act as scavengers of free radicals. Effectively inactivate singlet oxygen, and also react with organic free radicals produced during the process of lipid peroxidation [18]. In our study, total carotenoid and ascorbic acid contents in the camellia mistletoe extracts ranged from 1.7 to 4.4 mg/100 g and from 0.4 to 1.4 mg AA/100 g, respectively (Table 1). The methanol extract of camellia mistletoe had significantly higher TCC than the ethanol extract, whereas the lower amount of L-ascorbic acid was found in the ethanol extract of *Camellia* mistletoe (p<0.05) (Table 1). This result may be due to the solubility of ascorbic acid and carotenoid compounds. The carotenoid and ascorbic acid contents of the European mistletoe (*V. album*) have been reported to be 0.2-0.6 mg/100 g and 0.12-0.14 mg AA/100 g, respectively [19].

Antioxidant activities

Free radicals are involved in many disorders such as inflammation, heart disease, diabetes, gout, and cancer [20]. Antioxidants, due to their scavenging activity, are useful for the management of these diseases. As seen in Table 2, the methanol extract (IC_{50}=0.5 mg/mL) of *Camellia* mistletoe possess significantly higher DPPH scavenging activity than that of the ethanol extract (IC_{50} = 0.7 mg/mL) (p<0.05). However, the methanol extract showed similar scavenging activities for superoxide and nitric oxide radicals as ethanol extract (Table 2).

Iron is essential for life as it is required for oxygen transport, respiration and activities of many enzymes. Chelating agents inhibit lipid peroxidation by stabilizing the transition metals [21]. Decrease

Table 1: Total flavonoid, carotenoid and L-ascorbic acid contents of the methanol and ethanol extracts from *Camellia* mistletoe (*K. japonica*)

Solvent	Total flavonoid (mg RE/100 g)	Total carotenoid (mg/100 g)	L-ascorbic acid (mg AA/100 g)
100% MeOH	20.3±0.76*	4.4±0.02*	0.4±0.09*
70% EtOH	14.9±0.16	1.7±0.01	1.4±0.13

RE: Rutin equivalent, AA: L-ascorbic acid, *Values of 100% MeOH are significantly different from corresponding 70% EtOH (p<0.05). *K. japonica: Korthalsella japonica*

in the red color ferrozine–Fe^{2+} complex indicates higher scavenging activity of the compound. The metal chelating ability of the methanol and ethanol extracts of the *Camellia* mistletoe was represented in Fig. 1. The chelating effect of the extracts increases with the increase in concentration. Chelating effect on ferrous ions of the *Camellia* mistletoe extracts was 73-95% at 1.0 mg/mL. The IC_{50} value for the chelating effect of the methanol extract was 0.5 mg/mL, which was higher than that of the ethanol extract (0.4 mg/mL)($p<0.05$)(Fig. 1). EDTA used as positive control showed 99% at 0.5 mg/mL. The results suggest that the *Camellia* mistletoe extracts are capable of scavenging free radicals and prevent the initiation of free radicals by stabilizing them to participate in any deleterious reactions.

Table 2: IC_{50} value in free radical scavenging property of the methanol and ethanol extracts of *Camellia* mistletoe (*K. japonica*)

Solvent	IC_{50} value (mg/mL)		
	DPPH	Superoxide anion	Nitric oxide
100% MeOH	0.5±0.001*	0.4±0.03	0.7±0.01
70% EtOH	0.7±0.003	0.4±0.02	0.7±0.02

IC_{50} was obtained by interpolation from linear regression analysis; each value is expressed as mean±standard deviation (n=3). *Value is significantly different from corresponding 70% EtOH ($p<0.05$). *K. japonica: Korthalsella japonica*

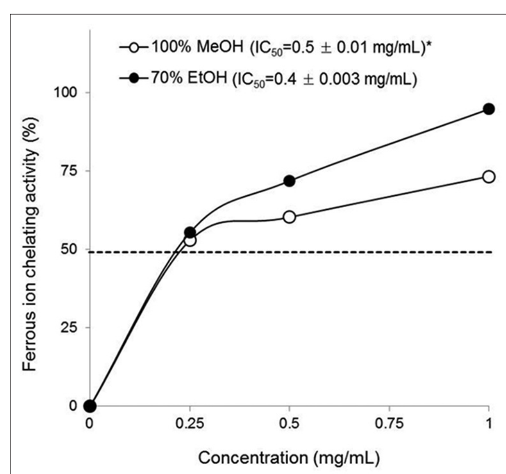

Fig. 1: Ferrous ion chelating ability of the methanol and ethanol extracts of obtained from *Camellia* mistletoe (*Korthalsella japonica*), each value is expressed as mean±standard deviation (n=3)and p<0.05, compared to 70% EtOH

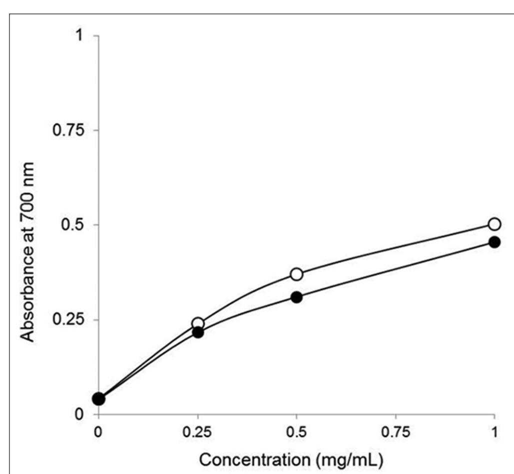

Fig. 2: Reducing power ability of the methanol and ethanol extracts obtained from *Camellia* mistletoe (*Korthalsella japonica*), each value is expressed as mean±standard deviation (n=3)

The conversion of Fe^{3+} to Fe^{2+} in the presence of extract and fractions was measured to determine the reducing power. The reducing properties are generally associated with the presence of reductones (antioxidants), which have been shown to exert antioxidant action by breaking the free radical chain [22]. The antioxidant constituents of the extract or fractions caused reduction of ferric-cyanide complex to the ferrous form due to hydrogen donation from phenolic compounds [22]. In this study, reducing the power of the *Camellia* mistletoe extracts was determined using ascorbic acid as the positive control. The reducing ability increased with concentration (Fig. 2). The maximum absorbance for crude extract was 0.43-0.47 at 1.0 mg/mL compared to 0.64 of ascorbic acid at 0.25 mg/mL used as positive control.

CONCLUSION

Camellia mistletoe extracts have potent antioxidant properties and can be considered as a good source for the food industry and medicinal applications. Further, the phytochemical analysis is needed to isolate the compounds responsible for the wide spectrum of pharmacological activities.

ACKNOWLEDGMENT

This work was supported by Basic Science Research Program (2016R1A6A1A03012862 and 2014R1A1A2056292) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, and by Business for Cooperative R&D between Industry, Academy, and Research Institute (Grants No. C0345499) funded Korea Small and Medium Business Administration in 2015, Republic of Korea.

REFERENCES

1. Ishizu T, Winarno H, Tsujno E, Morita T, Shibuya H. Indonesian Medicinal Plants. XXIV. Stereochemical structure of perseitol-K^+ complex isolated from the leaves of *Scurrula fusca* (*Loranthaceae*). Chem Pharm Bull 2002;50:489-92.
2. Kim MK, Yun KJ, Lim DH, Kim JJ, Jang YP. Anti-inflammatory properties of flavone di-*C*-glycosides as active principles of camellia mistletoe, *Korthalsella japonica.* Biomol Ther 2016;19:1-8.
3. Osadebe PO, Okide GB, Akabogu IC. Study on anti-diabetic activities of crude methanolic extracts of *Loranthus micranthus* (Linn.)sourced from five different host trees. J Ethnopharmacol 2004;95(2-3):133-8.
4. Büssing A, Schietzel M. Apoptosis-inducing properties of *Viscum album* L. extracts from different host trees, correlate with their content of toxic mistletoe lectins. Anticancer Res 1999;19:23-8.
5. Zuber D. Biological flora of central Europe: *Viscum album* L. Flora 2004;199:181-203.
6. Lee CB. Korea flora. Seoul: Hyangmunsa; 1985. p. 295-6.
7. Hajtó T, Hostanska K, Berki T, Pálinkás L, Boldizsár F, Németh P. Oncopharmacological perspectives of a plant lectin (*Viscum album* Agglutinin-I): Overview of Recent Results from *In vitro* Experiments and *In vivo* Animal Models, and Their Possible Relevance for Clinical Applications. Evid Based Complement Alternat Med 2005;2(1):59-67.
8. Kang DH, Park EM, Kim JH, Yang JW, Kim JH, Kim MY. Bioactive compounds and antioxidant activity of Jeju camellia mistletoe (*Korthalsella japonica* Engl.). J Life Sci 2016;26:1074-81.
9. Kang DH, Kim MY. Antimicrobial activity of Korean camellia mistletoe *(Korthalsella japonica* (Thunb.)Engl.)extracts. J Appl Pharm Sci 2016;6:226-30.
10. Aharmas S, Saxena J. Phytochemical screening and quantitative estimation of total phenolic content and totoal flavonoid content of grains of *Paspalum scrobiculatum*. Asian J Pharm Clin Res 2016;9:73-6.
11. Rainha N, Lima E, Baptista J, Rodrigues C. Antioxidant properties, total phenolic, total carotenoid and chlorophyll content of anatomical parts of *Hypericum foliosum*. J Med Plants Res 2011;5:1930-40.
12. Klein BP, Perry AK. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. J Food Sci 1982;47:941-5.
13. Huang YC, Chang YH, Shao YY. Effects of genotype and treatment

on the antioxidant activity of sweet potato in Taiwan. Food Chem 2006;98:529-38.
14. Robbins RJ. Phenolic acids in foods: An overview of analytical methodology. J Agric Food Chem 2003;51(10):2866-87.
15. Atina RC, Irda F, Komar R. Comparison of five antioxidant assays for estimating antioxidant capacity from three *Solanum* SP. extracts. Asian J Pharm Clin Res 2016;9:123-8.
16. Vicas SI, Rugina D, Leopold L, Pintea A, Socaciu C. HPLC fingerprint of bioactive compounds and antioxidant activities of *Viscum album* from different host trees. Not Bot Horti Agrobot Cluj Napoca 2011;39:48-57.
17. Genet S, Kale RK, Baguer NZ. Alteration on antioxidant enzymes and oxidative damage in experimental diabetic rat tissue: Effect of vanadate and fenugreek (*Trigonella faenum graeoum*). Mol Cell Biochem 2002;236:7-12.
18. Sikora E, Cieślik E, Topolska K. The sources of natural antioxidants. Acta Sci Pol Technol Aliment 2008;7:5-17.
19. Barbasz A, Kreczmer B, Rudolphi-skórska E, Sieprawska A, Wožnica D. Content of antioxidants in extracts of mistletoe (*Viscum album* L.), yew (*Taxus baccata* L.), pine (*Pinus sylvestris* L.)and fir (*Abies alba* Mill.). Herba Pol 2012;58:27-36.
20. Slater TF. Free-radical mechanisms in tissue injury. Biochem J 1984;222(1):1-15.
21. Serargui F, Baghiani A, Khennouf S, Arrar L. Antioxidant activity assessment of *Tamus communis L*. Roots. Int J Pharm Pharm Sci 2016;8:64-71.
22. Vichit W, Nisakorn S. Antioxidant activities and cytotoxicity of Thai pigmented rice. Int J Pharm Pharm Sci 2015;7:329-34.