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Research Article

Effect of Extraction Solvent and Harvest Time on Recovery of Bioactive Compounds, Antioxidant and Cancer Cell Growth Inhibition Activities of Korean *Camellia mistletoe*

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ABSTRACT

This study aimed to determine the phytochemical profiles, *in vitro* antioxidant and antiproliferative properties of Korean *Camellia mistletoe* (*Korthalsella japonica* (Thunb.) Engl.) depending on the harvest time (August and November) and solvent (100% methanol, 70% ethanol, and hot water). The *Camellia japonica* L. mistletoe extracts were analyzed for total phenol, flavonoid, carotenoid, and L-ascorbic acid contents and antioxidant properties such as scavenging capacities (1,1-diphenyl-2-picrylhydrazyl (DPPH) and NO•), ferrous ion chelating and reducing power. Concurrently, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to assess the antiproliferative properties against human cancer cell lines; MCF (human breast cancer cells), HeLa (human cervical cancer cells), A375 (human malignant melanoma cells), HCT116 (human colon cancer cells), HepG2 (human liver cancer cells) and A549 (human non-small cell lung adenocarcinoma cells). The results showed that extraction solvent and harvest time had significant impacts on antioxidant and anticancer activities and selectivity for free phenolic compounds in *Camellia japonica* L. mistletoe. Among all the tested extracts, the highest amounts of total phenolic and total flavonoids content were found in ethanol extracts of *Camellia japonica* L. mistletoe harvested in November, while the methanol extracts of *Camellia japonica* L. mistletoe collected in August showed the highest contents of total carotenoids and L-ascorbic acids compared to the other tested extracts. Additionally, the highest NO• radical scavenging activity was found in ethanol extracts, whereas the strongest DPPH radical scavenging activity was found in methanol extracts of *Camellia japonica* L. mistletoe harvested in November. Furthermore, methanol extracts showed much higher antiproliferative activity against all human cancer cells than ethanol extracts of *Camellia japonica* L. mistletoe harvested in November. In conclusion, the antioxidant and anticancer properties of *Camellia japonica* L. mistletoe showed significant dependence on the extraction solvent type and harvest time. With optimum harvest time and extraction solvent, they boast a wide range of promising medical, pharmaceutical, and food applications.

Keywords: Anticancer activity, Antioxidant activity, Camellia mistletoe, Harvest time, Solvent

Introduction

Many plant extracts exhibit efficient antioxidant properties due to their phytoconstituents, such as phenolics, especially phenolic acids, flavonoids, and carotenoids [1]. The study of antioxidant compounds has drawn the interest of researchers because they are effective in inhibiting free radicals and thus decelerating the formation of degenerative disease [2-4]. Therefore, methods of extracting phenolic compounds from the source material are a major focus of investigation [5]. Current isolation and chemical purification meth-

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ods include solvent extraction processes that utilize solvent polarity as a major separation technique [6-7]. The use of solvents in a material must be based on the solution's solubility and the components' material to dissolve [6-7]. Phenolic components can be extracted from plant material using solvents such as water, methanol, ethanol, ethyl acetate, and phenol/chloroform [6-7].

Plant phytochemical accumulation is influenced by various external factors that change with harvest time [8-11]. The harvest time of plant products directly affects the composition and concentration of bioactive compounds and minerals by changing growth and weather conditions to which plants are exposed [8-11]. Previous studies showed that plant samples of the same species collected at different seasons or with different harvest times might have significant differences in their phytochemical and pharmacological properties [8-11]. However, there needs to be an adequate report regarding the effect of seasonal variation and extraction solvents on the bioactivity of *Camellia mistletoe*.

Mistletoes are evergreen and semi-parasitic plants growing on various trees and shrubs [12]. Mistletoes have recently been described as 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 [12]. *Camellia mistletoe, Korthalsella japonica* (Thunb.) Engl., is one of the Korean mistletoes species which is distributed in Jeju island, Korea [13]. They have been used in treating and managing many diseases for many years, both traditional and complementary medicine, such as diarrhea, cough, diabetes, hypertension, and cancer [2, 11-16]. Phenolics and flavonoids present in mistletoe are largely responsible for its biological effects, including anti-radical activity [2, 11-16]. It has been suggested that pharmacologically active compounds may pass from various host trees to the parasitic mistletoe plants [2, 11-16]. Therefore, it is important to develop a method for acquiring extracts with a high content of biologically active compounds.

To ensure high production and accumulation of desired bioactive compounds, it is necessary to identify the optimal harvest time and extraction solvent type for the plant. However, the influence of harvest time and extraction solvent on phenolic composition, antioxidant activity, and anticancer effects of *Camellia japonica* L. mistletoe has not been studied. Here, we investigated a possible correlation between harvest time, extraction solvent, and phenolic compound contents in *Camellia japonica* L. mistletoe with antioxidant and anticancer activities. The aim of this work was to evaluate the changes in the phenolic compound contents, antioxidant activity, cancer cell growth inhibition, and their relationship in methanol, ethanol, and water extracts of *Camellia japonica* L. mistletoe samples harvested at two different periods.

Material and Methods

Plant sample preparation

Camellia mistletoe (*Korthalsella japonica* (Thunb.) Engl.) samples were collected during August and November 2019 from Camellia Hill located in Andeok-myeon, Seogwipo-si, Jeju-do Province, South Korea and the voucher specimens (KHUP-0803) have been deposited in our laboratory for future reference. To prepare the solvent extracts of *Camellia mistletoe*, samples were freeze-dried. The ground powder of samples was then extracted at room temperature twice by constant shaking for 72 h with solvents (1:10, w/v) of various polarities including methanol (100%), ethanol (70%) and hot water. The solutions were then filtered through Whatman paper and concentrated in vacuo using a rotary evaporator (Buchi Rotavapor R-200, New Castle, DE, US). The yield of solvent extracts was determined and residues were the taken to a laboratory for further analysis.

Total phenol and flavonoid contents

Total soluble phenolics were spectrophotometrically determined with Folin-Ciocalteu reagent (Sigma-Aldrich, MO) using gallic acid as the standard, as reported previously [17]. Total phenolic content was calculated as gallic acid equivalents (GAE) per liter of *Camellia mistletoe* extracts based on comparison with a standard curve of gallic acid.

Total flavonoid content was determined by an aluminum chloride colorimetric method [17]. Fifteen microliters of *Camellia mistletoe* extracts were 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 solution was well mixed, and the absorbance was

measured immediately against the prepared blank at 510 nm compared with the standards prepared similarly with known rutin concentrations. The content of flavonoids was calculated based on the calibration curves of rutin, and the results were expressed as the mg of rutin equivalents (RE) per liter of sample.

Free radicals (DPPH and nitric oxide) scavenging activities

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the *Camellia mistletoe* extracts was measured according to the modified methods [17]. Briefly, freshly prepared 100 µL of 0.4 mM DPPH solution dissolved in methanol was added to the equal volume of each sample fraction. The reaction mixture was incubated for 10 min and the absorbance was measured at 517 nm using a Spectra MR microplate reader (Dynex Technologies, Inc., Chantilly, VA, US). The commercial known antioxidant, butylatedhydroxytoluene (BHT) was used as a positive control.

Nitric oxide radical (NO˙) inhibition was measured by the Griess reduction [18]. Sodium nitroprusside in phosphate-buffered saline (10 mM, pH 7.0) was added to the extracts and the mixtures (100 µL) were incubated at 25°C for 3 h. Then, an equal volume of Greiss reagent was addded and kept for 5 min. The absorbance of these solutions was measured at 540 nm. BHT was used as a positive control.

Ferrous ion chelating activity

The chelating ability was determined according to the method of Kim *et al.* [17]. A volume of 5 µL of freshly prepared $FeCl_2$ (2 mM) was mixed with 250 µL of *Camellia mistletoe* extracts or ethylenediaminetetraacetic acid (EDTA, positive control). A 10 µL of 5 mM ferrozine was added to the mixture and absorbance readings were taken after exactly 10 min at 25 ºC.

Reducing power activity

The Fe^{3+}reducing power of *Camellia mistletoe* extracts was carried out as described previously developed method of Moon and Kim [14]. Different concentrations of the extract (200 µL, 0.125-2 mg/mL) were mixed with 200 µL of 200 mM phosphate buffer (pH 6.6) and $K_3Fe(CN)_6$ (200 µL, 1%). After incubation for 20 min at 50°C, 200 µL of 10% trichloroacetic acid solution was added to the mixture, and then centrifuged at 800 × g for 10 min. The upper layer of solution (100 µL) was mixed with deionized water (100 µL) and $FeCl_3$ solution (20 µL, 0.1%) prior to measuring the absorbance at 700 nm.

Cell culture

Anticancer activity of *Camellia mistletoe* extracts was determined against a panel of human cancer cell lines of MCF7 (breast), HeLa (cervical), A375 (melanoma), HCT 116 (colon), HepG2 (liver), A549 (lung), besides one normal cell line (TK6). Cells were grown in DMEM (MCF7, HeLa and A375), McCoy's 5A (HCT 116), MEM (HepG2), Ham's F-12 (A549) and RPMI 1640 (TK6) supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin and 2 mM L-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 ºC.

Cytotoxicity assay

Cell viability was examined by the mitochondrial activity of the cells to cleave the tetrazolium salt the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) according to a previously described method [19]. Briefly, each extract was presolubilized in dimethylsulphoxide (DMSO) at 37°C to give a stock solution (1 mg/mL). Serial ten-fold dilutions were made from the stock solution to give working concentrations of 0-200 µg/mL. The final concentration of DMSO to which cells mL were exposed was less than 1%. Confluent monolayers of cells were grown in 96-well culture plates for 24 h. Cells were incubated with various test extracts in triplicate concentrations at 37°C in a CO_2 environment for 72 h. The negative control was performed using a growth medium alone instead of plant extract. At the expiration of the 72 h treatment period, supernatants were removed from the wells, and 25 µL of the MTT solution (2 mg/mL in PBS) was added to each well. The plates were incubated for 4 h at 37°C, and 125 µL of DMSO was added to each well to dissolve the formazan crystals. The optical density was determined at 540 nm using a Spectra MR microplate reader. The 50% inhibition of cell proliferation (IC_{50}) was defined as the extract concentration required to reduce cell viability by half.

Statistical analysis

All data are presented as mean ± standard deviation of triplicate values. Significant differences between the groups were performed by using SPSS program (SPSS Inc. Chicago, IL, USA) using two-tailed Student's *t*-test. A *p*-value less than 0.05 was considered statistically significant.

Results and Discussion

Influence of extraction solvents and harvest time on bioactive compounds

Phenolic compounds are a class of antioxidant agents, which can quench and neutralize the free radicals [1-4, 18]. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids are the biggest secondary metabolites of phenolic compounds in plants with more than 4,000 individual identified compounds [1, 20-21]. Flavonoids have been linked with various health benefits [1, 20-21], thus, it is necessary to determine the impact of solvents on extraction yields of flavonoids for their maximum extraction. Sample content of total phenolic compounds (mg GAE/100 g) and flavonoid (mg RE/100 g) in different solvent extracts of *Camellia mistletoe* harvested in August and November are summarized in Figure 1. In the current study, the variation obtained in total phenolic and flavonoid contents depend on the harvest time as well as the extraction solvents (Figure 1). Results of the assays for phenolics described in the present report, indicated a wide variation in the total phenolic content in the different extracts, ranging from 22359 to 37628 mg GAE/100 g of extract. Results of these assays, demonstrated significant variability ($p < 0.05$) in total yield of phenolic compounds depend on solvent and harvest time.

Different solvents will extract different classes of polar metabolites present in plant sample. In the present study, ethanol proved to be the most effective solvent for isolation of phenolic compounds (22359-64485 mg GAE/100 g) from samples of *Camellia mistletoe*, whereas much lower yields were obtained from samples extracted with hot water (7635-37628 mg GAE/100 g) (Figure 1A). The order of effectiveness in extraction of phenolics from *Camellia mistletoe* harvested in August and November was ethanol>methanol>water (Figure 1A).

The amounts of total flavonoids extracted from *Camellia mistletoe* harvested in August and November using different solvents type ranged from 3298 to 20109 mg RE/100 g and from 4806 to 23988 mg RE/100 g, respectively, with the highest amounts of total flavonoids observed in the ethanol extract (23988 mg RE/100 g) of *Camellia mistletoe* harvested in November, whereas the lowest yield was obtained in the water extract (3298 mg RE/100 g) of *Camellia mistletoe* harvested in August (Figure 1B).

On the contrary, methanol is mostly indicated for the extraction of carotenoids and ascorbic acid (Table 1). Among the solvents, methanol had the highest recovery with carotenoids (27476 mg/100

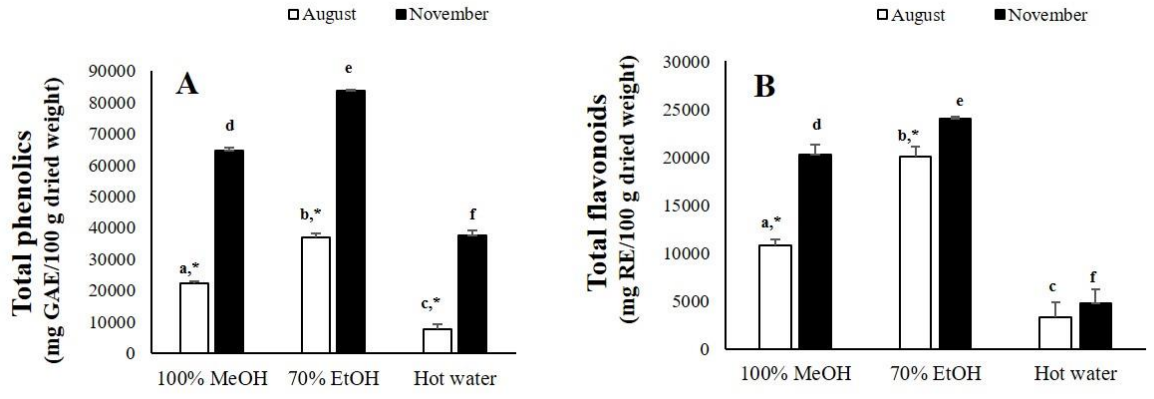

Figure 1. Total phenolic (A) and flavonoid (B) compositions of Korean *Camellia mistletoe* extracts. Values are the mean of three replications (n=3). [a-f] Values with different superscripts in a column ([a-c] August or [d-f] November) are significantly different ($p < 0.05$). [*] $p < 0.05$ compared to November by Student's *t*-test.

Table 1. The extraction yield (%) of Korean *Camellia mistletoe* extracts

Solvent	August	November
100% MeOH	15.7 ± 1.49	16.7 ± 0.74
70% EtOH	18.3 ± 1.16	20.3 ± 1.03
Hot water	19.5 ± 2.13	14.4 ± 1.67

Note: Each value is expressed as mean ± standard deviation (n = 3).

Table 2. Carotenoid and L-ascorbic acid contents of Korean *Camellia mistletoe* extracts

Harvest time	Solvent	Total carotenoid (mg/100 g)	L-Ascorbic acid (mg/100 g)
August	100% MeOH	$27476 \pm 125^{a,*}$	$1385 \pm 7^{a,*}$
	70% EtOH	$11693 \pm 3^{b,*}$	$960 \pm 21^{b,*}$
	Hot water	6589 ± 53^{c}	271 ± 20^{c}
November	100% MeOH	15468 ± 154^{d}	1007 ± 24^{d}
	70% EtOH	8646 ± 55^{e}	707 ± 7^{e}
	Hot water	6279 ± 28^{f}	217 ± 18^{f}

Note: Each value is expressed as mean ± standard deviation (n = 3). [a-f] Values with different superscripts ([a-c] August or [d-f] November) are significantly different ($p < 0.05$). [*] $p < 0.05$ compared to November by Student's *t*-test.

g) and ascorbic acid (1385 mg/100 g) from *Camellia mistletoe* harvested in August. Methanol was closely followed by ethanol with about 42.6 and 69.3% of carotenoids and ascorbic acid being recovered in comparison with methanol. Water had the lowest recovery yields of carotenoids and ascorbic acid with less than 23.9 and 19.6% in comparison with methanol (Table 2). Samples harvested in August had significantly higher contents of carotenoids and ascorbic acid than those harvested in November (Table 2). Carotenoids, vegetable dyes present in the chloroplasts and chromophores, play an auxiliary role in the process of photosynthesis [22]. Vegetable carotenoids have antioxidant activity and can prevent vitamin A deficiencies [23]. Ascorbic acid is an antioxidant vitamin that acts synergistically with tocopherol to preserve antioxidant function in chronic disease states [24]. Ascorbic acid also has a protective function against photo oxidation processes [25].

Our findings indicate that the extraction solvent and harvest time play an important role in extractability of bioactive compounds from the plants. This study recommends that 70% of ethanol should be used for extraction of total phenolic compounds and flavonoids (Figure 1), and 100% of methanol should be used for extraction of total

Table 3. IC_{50} value (mg/mL) in antioxidant activity of Korean *Camellia mistletoe* extracts

Harvest time	**Solvents**	**Radical scavenging**		**Ferrous ion chelating**	**Reducing power**
		DPPH	**Nitric oxide**		
August	100% MeOH	$0.9 \pm 0.002^{a,*}$	1.6 ± 0.04^{a}	0.4 ± 0.04^{a}	1.0 ± 0.01^{a}
	70% EtOH	$1.2 \pm 0.02^{b,*}$	1.2 ± 0.12^{b}	0.3 ± 0.01^{a}	1.1 ± 0.01^{a}
	Hot water	$2.5 \pm 0.47^{c,*}$	$3.2 \pm 0.90^{c,*}$	0.3 ± 0.01^{a}	$2.9 \pm 0.01^{b,*}$
November	100% MeOH	0.6 ± 0.003^{d}	1.5 ± 0.10^{d}	0.4 ± 0.05^{d}	0.9 ± 0.01^{d}
	70% EtOH	0.8 ± 0.03^{e}	1.1 ± 0.07^{e}	0.3 ± 0.0^{d}	1.1 ± 0.01^{d}
	Hot water	0.7 ± 0.06^{d}	1.7 ± 0.07^{d}	0.3 ± 0.01^{d}	1.8 ± 0.02^{e}

Note: IC_{50} means the effective concentration at which the antioxidant activity was 50%, which was obtained by interpolation from linear regression analysis. Each value is expressed as mean ± standard deviation (n = 3). [a-f] Values with different superscripts ([a-c] August or [d-f] November) are significantly different ($p < 0.05$). [*] $p < 0.05$ compared to November by Student's *t*-test.

carotenoid and L-ascorbic acid (Table 2). The extracting solvent also affects the number of active compounds contained in the extract, according to the concept of like dissolve like, where polar compounds will dissolve in polar solvents, and non-polar compounds will dissolve in non-polar solvents. A solvent such as methanol and ethanol are very widely used solvents and are sufficient for the extraction of phenolic components from natural materials.

Influence of extraction solvents and harvest time on Antioxidant activities

The antioxidant capacity of the different extracts of *Camellia mistletoe* collected in two different seasons was investigated using free radical scavenging, ferrous ion chelating and reducing power assays (Table 3). The inhibitory concentration (IC_{50}) values were determined from plotted graphs and indicated the amount of test sample needed to inhibit or scavenge 50% of the radicals present in the reaction mixture. As shown in Table 2, the antioxidant activity was significantly higher ($p < 0.05$) in samples collected in November than in those harvested in August.

Many studies have reported differences in the bioactivity of the same plant collected in different seasons. Nakanishi *et al.* [26] have reported that the α-glucosidase inhibitory activity of mulberry extracts diverse depend on the environmental condition of temperature and photoperiod. Son and Lee [27] also demonstrated seasonal variation in α-glucosidase inhibitory activity of *Cudrania tricuspidata* extracts undergoes seasonal variation with the highest level of activity obtained from autumn to winter.

The harvesting time of plant products directly affects the composition and concentration of bioactive compounds and minerals by changing growth and weather conditions to which plants are exposed [28]. Previous studies showed a close relationship between phytochemical composition and concentration and compound activity in plants [11]. Plant samples of the same species collected at different harvesting times may have significant differences in their phytochemical and pharmacological properties [28-31]. The impact of the mistletoe collection period on the chemical composition and biological activity of extracts was studied previously [32]. The authors studied the total phenol content and antioxidant activity of aqueous and methanol extracts from leaves and stems of mistletoe in May, July and December [32]. They found that the differences in the antioxidant activity of mistletoe harvested from different trees and in different seasons can be attributed to factors such as climate and temperature, which can significantly affect the chemical composition and antioxidant activity of plants [32]. Recently, we also shown that a considerable seasonal variation of inhibition of α-glucosidase, lipase, tyrosinase and $NO^{\bullet}$ production was observed in *Camellia mistletoe* extracts [33].

Methanol and ethanol solvents gave significantly higher properties of DPPH- and nitric oxide-scavenging, ferrous ion chelating and reducing power than water solvent (Table 2). Among the investigated extracts, methanol extract of *Camellia mistletoe* harvested in November exhibited maximum DPPH radical scavenging activity (Table 3). All extracts showed significantly lower DPPH radical scavenging activity than that of BHT standard. Particularly, the IC_{50} value of BHT was 0.027 μg/mL, while the IC_{50} value of methanol extract was 900 mg/mL (Table 3).

The role of nitric oxide in various disease states has attracted the attention of scientists worldwide. The nitric oxide does not interact with bioorganic macromolecules such as DNA or proteins directly [28]. However, under aerobic conditions, the nitric oxide molecule is very unstable and reacts with the oxygen to produce intermediates such as $NO_2^{\bullet}$, $N_2O_4^{\bullet}$ and $N_3O_4^{\bullet}$ and stable products like nitrate and nitrite and peroxynitrite when reacted with superoxide which is highly toxic to humans [28]. Results shown here indicate that ethanol solvent exhibits significantly higher nitric oxide scavenging activities than methanol and water extracts ($p < 0.05$) due to the high polarity of the solvent system (Table 3). The present study indicates that the *Camellia mistletoe* extracts have good scavenging activity for nitric oxide but is not as efficient as the standard BHT (IC_{50} = 75 μg/mL).

However, methanol and ethanol extracts showed similar metal chelating and reducing power abilities (Table 3). The IC_{50} value of the chelating effect of methanol and ethanol extracts was 0.4 and 0.3 mg/mL, respectively (Table 3). The IC_{50} value of standard EDTA were 1.27 μg/mL. Methanol and ethanol extracts also exhibited similar reducing power activities and low IC_{50} values; The IC_{50} values varied from 0.9 for methanol to 1.1 mg/mL for ethanol (Table 3).

Table 4. IC_{50} value (μg/mL) in anticancer activity of Korean *Camellia mistletoe* extracts

Harvest time	Solvent	TK6	MCF7	HeLa	A375	HCT116	HepG2	A549
August	100% MeOH	100.2 ± 3.98 [a]	41.2 ± 0.90 [a,*]	44.3 ± 1.46 [a]	52.7 ± 3.33 [a]	75.1 ± 1.87 [a]	56.7 ± 8.59 [a]	154.0 ± 4.91 [a,*]
	70% EtOH	113.7 ± 4.47 [a]	85.5 ± 1.24 [b,*]	53.9 ± 5.49 [a,*]	83.0 ± 1.93 [b,*]	90.1 ± 1.00 [b,*]	105.9 ± 3.54 [b,*]	209.5 ± 8.37 [b,*]
	Hot water	108.1 ± 3.85 [a]	98.8 ± 5.39 [c,*]	167.5 ± 2.25 [b,*]	94.5 ± 1.45 [c,*]	200.0 ± 5.43 [c,*]	193.1 ± 17.36 [c,*]	374.4 ± 8.82 [c,*]
November	100% MeOH	105.3 ± 3.26 [d]	41.1 ± 1.54 [d]	46.7 ± 1.20 [d]	45.4 ± 2.52 [d]	66.9 ± 1.47 [d]	67.8 ± 3.81 [d]	170.0 ± 8.36 [d]
	70% EtOH	110.4 ± 8.52 [d]	45.5 ± 2.13 [e]	52.2 ± 3.96 [d]	49.7 ± 6.52 [d]	79.4 ± 3.63 [e]	47.5 ± 3.73 [e]	129.4 ± 6.40 [e]
	Hot water	97.6 ± 5.75 [d]	76.8 ± 4.42 [f]	159.2 ± 11.63 [e]	157.0 ± 1.28 [e]	176.2 ± 4.15 [f]	105.6 ± 3.80 [f]	238.8 ± 2.75 [f]

Note: IC_{50} means the effective concentration at which the antioxidant activity was 50%, which was obtained by interpolation from linear regression analysis. Each value is expressed as mean ± standard deviation (n = 3). [a-f] Values with different superscripts ([a-c] August or [d-f] November) are significantly different ($p < 0.05$). [*] $p < 0.05$ compared to November by Student's *t*-test. Paclitaxol, a positive control, exhibited cytotoxicity with the IC_{50} values of 0.45, 108.48, 86.17, 1.92, 3.38 and 2.01 μg/mL against MCF7, HeLa, A375, HCT116, HepG2 and A549 cell lines, respectively.

The scavenging potentials of antioxidant components are closely associated with their total phenolic and flavonoid contents (Figure 1). The extracts with higher levels of total phenolics and flavonoids, also exhibit greater ferric chelating and reducing power activities. Variations in phenolic compounds content and antioxidant capacity between extracts were probably related to polarity of the extracting solvent.

Influence of extraction solvents and harvest time on human cancer cell viability and cell growth

Because of serious side effects and toxicity of chemotherapeutic anticancer agents, many cancer patients seek alternative and/or complementary methods of treatment. Plants have been used for treating various diseases of human beings and animals since time immemorial. They maintain the health and vitality of individuals, and also cure diseases, including cancer without causing toxicity. More than 50% of all modern drugs in clinical use are of natural products, many of which have the ability to control cancer cells [34]. Medicinal plants are therefore being investigated for their anticancer properties, and the demand for natural anticancer agents increasing. In the present study, the cytotoxicity of *Camellia mistletoe* extracts at the different concentrations was investigated on the MCF (human breast cancer cells), HeLa (human cervical cancer cells), A375 (human malignant melanoma cells), HCT116 (human colon cancer cells), HepG2 (human liver cancer cells), A549 (human non-small cell lung adenocarcinoma cells) and TK6 (human normal lymphoblastoid cells) to determine the IC_{50} value (μg/mL) that causes 50% cell death (Table 4). The cancer cell growth inhibition measured using MTT assay was significantly higher in November collected *Camellia mistletoe* sample than in those collected in August (Table 4). Our results also demonstrated that the percentage of viable cells changed according to the cell lines used (Table 4). The viability of all the cancer cells was significantly reduced by *Camellia mistletoe* extracts in a concentration-dependent manner.

For the development of anticancer agents from natural products, it is important not only to screen the selectivity (or specificity) of the natural products among several cancer cells but also to assay if natural products exhibited any cytotoxicity in non-cancer cells. Thus, the effect of *Camellia mistletoe* extracts on non-cancer cells (human fibroblast TK6 cells) was tested. The TK6 cells did not exhibit cytotoxic effect with any extracts (Table 4). One drawback of cytotoxic drug therapy for treatment of malignant diseases is serious toxicity. The data described above suggested that these *Camellia mistletoe* extracts are expected to be candidates for a non-toxic antitumor agent.

The highest cytotoxic activity (IC_{50} of 41 μg/mL) was obtained against the MCF7 cell line by the methanol extract of *Camellia mistletoe* (Table 4). The positive control, paclitaxol, which

is a known anti-cancer drug, resulted in high cytotoxicity against MCF7 cells with IC_{50} value of 0.45 µg/mL. The cytotoxicity, with an IC_{50} value varied from 41 to 68 µg/mL, was observed in methanol extracts of *Camellia mistletoe* harvest in November against HeLa, A375, HCT116 and HepG2 cells, whereas methanol extracts of *Camellia mistletoe* harvest in November exhibited the lowest cytotoxic effect on A549 cells, with an IC_{50} value of 170 µg/mL (Table 4). One drawback of cytotoxic drug therapy for treatment of malignant diseases is serious toxicity. The data described above suggested that these *Camellia mistletoe* extracts are expected to be candidates for a non-toxic antitumor agent.

Conclusion

Results of this study demonstrated that harvest time and type of solvents used significantly affected the extraction efficiency of bioactive compounds, antioxidant and anticancer potencies of *Camellia mistletoe*. The obtained data also showed that methanol and ethanol was the efficient solvents for extraction of the highest amounts of bioactive compounds, antioxidant and anticancer capacities of *Camellia mistletoe*. Our results revealed that *Camellia mistletoe* extracts are a potential source of active compounds for the development of natural drugs against cancer.

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