

Original Article

INHIBITORY ACTIVITIES OF *CAMELLIA* MISTLETOE (*K. JAPONICA*) EXTRACTS ON PANCREATIC LIPASE, TYROSINASE AND CANCER CELL PROLIFERATION

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ABSTRACT

Objective: The objective of this study was undertaken to examine pancreatic lipase and tyrosinase inhibitory activities of *Camellia* mistletoe (*Korthalsella japonica*) extracts and conduct their antiproliferative activity on several human cancer (Hela, A375, HCT 116, HepG2 and A549) and normal cells (TK6) lines.

Methods: The lipase inhibitory activity of acts of *Camellia* mistletoe extracts were determined from the hydrolytic reaction of p-nitrophenyl butyrate with pancreatic lipase and cytotoxicity against human cancer and normal cell lines were also evaluated using the MTT assay. The *in vitro* tyrosinase inhibitor potency of *Camellia* mistletoe extracts was estimated using mushroom tyrosinase.

Results: Both methanol and ethanol extracts of *Camellia* mistletoe exhibited an inhibitory effect on lipase inhibition activity with IC₅₀ values of 0.39 and 0.49 mg/ml, respectively. Moreover, *Camellia* mistletoe extracts were judged to be strongly active against the cancer cell lines, while they had no antiproliferative activity against the normal human cell lines. However, they had little inhibitory effects on tyrosinase activity.

Conclusion: *Camellia* mistletoe extracts could be the good sources of natural lipase inhibition and anticancer agents with a great potential to be used in food and therapeutic fields.

Keywords: *Camellia* mistletoe, Pancreatic lipase inhibitory activity, Tyrosinase inhibitory activity, Antiproliferative activity

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INTRODUCTION

Medicinal plants are an important therapeutic aid for various ailments. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems [1]. Plants are a rich source of medicines because they produce a wide array of bioactive molecules, most of which probably evolved as a chemical defence against predation or infection. The increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that in order to find active compounds, a systematic study of medicinal plants is very important. Compounds from medicinal plants in the food and cosmetics industry have a central role and have a wide range of applications.

Mistletoes are native to Asia, Africa, Europe and Australia, and 70-100 species have been recorded [2]. Mistletoe extracts are used for the treatment of many diseases such as cancer, hypertension, and diabetes [3-5]. *Camellia* mistletoe, *Korthalsella japonica* (family Santalaceae), is a semiparasitic plant which is distributed in Jeju Island, Korea. Recently there has been considerable interest in the antioxidant potential and antimicrobial activities of *Camellia* mistletoe extracts [6, 7]. Although a lot of work was carried out with regard to the phytochemical and pharmacological evaluation of European mistletoe, *Viscum album* L. [8], but little is known of actual physiological benefits of *Camellia* mistletoe, *K. japonica*. Therefore, the present study was aimed to evaluate inhibitory activities of lipase, tyrosinase and cancer cell proliferation by the methanol and ethanol extracts from *Camellia* mistletoe.

MATERIALS AND METHODS

Chemicals and reagents

Porcine pancreatic lipase, p-nitrophenyl butyrate (p-NPB), mushroom tyrosinase, tyrosine, ascorbic acid were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Cell culture reagents were obtained from Lonza (Walkersville, MD, USA). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) cell proli-

feration kit was purchased from Boehringer Mannheim (Indianapolis, IN, 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 [7]. The dried and powdered plant material (20 g) was extracted with 100 ml of 100% methanol or 70% ethanol for 72 h and filtered, 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. The average extraction yields (fresh sample/methanol or ethanol) were 7.1 and 10.4%, respectively. Dried extracts were reconstituted in dimethyl sulfoxide (DMSO, Amresco, Solon, Ohio, USA). DMSO acts as a solvent without changing any property of the methanol and ethanol extracts.

Pancreatic lipase inhibitory activity

The lipase inhibitory activity of *Camellia* mistletoe extracts was estimated from the ability to inhibit the *in vitro* porcine pancreatic lipase activity as previously described [9] with slight modifications. The reaction mixture consisted of 6 µL of 10 mg/ml of porcine pancreatic lipase and 170 µL of Tris buffer. Then 20 µL of *Camellia* mistletoe extracts was added and incubated at 37 °C for 15 min. Four microliters of 10 mM p-NPB was then added and incubated at 37 °C for 60 min.

Lipase activity was determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 400 nm using a Spectra MR microplate reader (Dynex Technologies, Inc., Chantilly, VA, US). IC₅₀, the concentration of a tested compound giving 50% inhibition of the enzyme activity, was evaluated from the least square regression line of the plot of the logarithm of concentration vs. the inhibitory activity. Hesperidin was used as the positive control [10].

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity of *Camellia* mistletoe extracts was carried out by a previously described method [11] with some modifications. Briefly, mushroom tyrosinase (40 μ l, 110 unit/ml) was mixed with 100 μ l of phosphate buffer (0.175 M, pH 6.8) and 40 μ l of 10 mmol tyrosine and 20 μ l of different concentrated *Camellia* mistletoe extracts (0.125, 0.25 and 0.5 mg/ml). The mixture was then incubated for 15 min at room temperature. Following incubation, absorbance of the mixture was determined at 475 nm by using a Spectra MR microplate reader. The concentration of the extract which caused a half maximal inhibition of the lipase activity (IC_{50}) was obtained from a semilog plot of *Camellia* mistletoe extract concentrations against the percentage of enzyme inhibition. Ascorbic acid (500 μ g/ml) as positive control was used for assay.

In vitro cytotoxic activity

The *in vitro* cytotoxic activity of *Camellia* mistletoe extracts was determined from the mitochondrial activity of cell lines which represent the number of viable cells after the treatment, by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cytotoxic assay on six different human cell lines in tissue culture. The human lymphoblastoid cells (TK6), cervical cancer (Hela), malignant melanoma (A375), colon cancer (HCT 116), liver cancer (HepG2) and non-small cell lung adenocarcinoma (A549) cell lines were kindly provided by Professor Wogan (Massachusetts Institute of Technology, MA, US). Hela and A375 cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μ g/ml streptomycin and 2 mmol L-glutamine at 37 °C with 5% CO₂ in a humidified atmosphere. TK6, HCT 116, HepG2 and A549 cell lines were maintained in RPMI 1640, McCoy's 5A, MEM and Ham's F-12 media supplemented as above with L-glutamine, antibiotics and FBS, respectively.

The inhibitory effect of *Camellia* mistletoe extracts on the proliferation of human normal and cancer cells was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cell suspensions (5 \times 10³ per well) were loaded into 96-well culture plates and treated with fresh medium containing various concentrations (0-200 μ M) of each extract for 72 h. Cells were washed once with phosphate buffered saline and reacted with the MTT solution at 37 °C for 4 h to produce the formazan salt. Finally, the formazan salt formed in each cultured cells was dissolved in DMSO, and the optical density (OD) value of each solution was measured at 540 nm using a Spectra MR microplate reader. The OD value detected for the control (cells without treatment with any samples) from the treated cells was plotted on the x-axis, designated as proliferation (% of control), to demonstrate the effect of each sample on the viability of the related cells. The concentration of the extract which caused a half-maximal inhibition of cell proliferation (IC_{50}), as determined by the MTT assay, was obtained from a semi-log plot of *Camellia* mistletoe extract concentrations against the percentage of cell survival.

Statistical analysis

Data were analyzed using SPSS statistical software (SPSS, ver. 12.0; SPSS Inc., Chicago, IL, US). Comparisons of all results between methanol and ethanol extracts of *Camellia* mistletoe were made by using a non-parametric test (Mann-Whitney U test) with $p < 0.05$.

RESULTS AND DISCUSSION

Lipase inhibitory activity

Obesity is now recognized as a major risk factor in the metabolic syndrome, diabetes mellitus, hypertension and some cancers [12]. Obesity is considered an extremely costly health problem which in developed nations accounts for 2-6% of total health care costs [13]. Currently Orlistat, a potent competitive inhibitor of gastric and pancreatic lipase, has been approved by FDA and is available as an antiobesity drug [14]. However, this medication is associated with incidence of side-effect including intestinal flatulence, borborygmi and abdominal cramps [15]. Therefore, new approaches for the treatment of obesity tried to reduce energy intake through gastrointestinal mechanisms, without altering any vital mechanisms [16]. In our current study, methanol and ethanol extracts of *Camellia* mistletoe inhibited the lipase activity in a dose-dependent manner in the assay system with p-NPB as a substrate (table 1). With respect to the lipase inhibition, the methanol extract showed more effective activity than ethanol extract ($p < 0.05$). Nevertheless, this was weak (IC_{50} value of 0.39 mg/ml), compared to that for the positive control of hesperidin (IC_{50} value of 0.032 mg/ml). However, the pancreatic lipase inhibitory effect of *Camellia* mistletoe was superior to those when various dietary plants were screened by Kim et al. [17] and Conforti et al. [18].

Tyrosinase inhibitory activity

Tyrosinase inhibitor has been used frequently in cosmetics and depigmenting agents for hyperpigmentation. It catalyzes two different reactions using molecular oxygen the ortho-hydroxylation of tyrosine (mono-phenols) named monophenolase activity and the oxidation of 3,4-Dihydroxyphenylalanine (DOPA) to dopaquinone (o-quinones) named diphenolase activity [19]. A large number of moderate to potent tyrosinase inhibitors from natural and synthetic resources have been reported during the last decade [20, 21]. However, some tyrosinase inhibitors are considered to be cytotoxic to melanocytes and potentially mutagenic to mammalian cells [21, 22]. Therefore, it remains necessary to search for new tyrosinase inhibitors without side effects. In this study, we found that *Camellia* mistletoe extracts fraction had a small inhibitory effect at the highest dose tested (table 1). However the inhibitory effect is small and it is not economically feasible to be developed further. Ascorbic acid, used as a positive control, reduced mushroom tyrosinase drastically even at the lowest dose used. Although the results of this work are inconsistent with those reported by Lee et al. [23] in that high tyrosinase inhibitory activity was detected from hot water (64%) and ethanol (63%) extracts of mistletoe (*Viscum album*) at the concentration of 100 mg/ml, our study was conducted with different extraction methods, different species (*K. japonica*) and relatively very low test concentrations (0.125, 0.25 and 0.5 mg/ml).

Table 1: Effects of the methanol and ethanol extracts from *Camellia* mistletoe (*K. japonica*) on lipase and tyrosinase inhibitory activities

	Solvent	Concentration (mg/ml)	Inhibition (%)	IC_{50} value (mg/ml)
Lipase inhibitory activity	100% MeOH	0.125	40.1 \pm 0.15M	0.39 \pm .395MeOH
		0.25	41.9 \pm 1.95M	
		0.5	53.3 \pm 3.3se	
	70% EtOH	0.125	14.9 \pm 4.95t	0.49 tOHnhibi
		0.25	25.9 \pm 5.9 t	
		0.5	50.5 tOHnhi	
Tyrosinase inhibitory activity	100% MeOH	0.125	10.8 \pm 0.85M	1.40 \pm .405MeO
		0.25	16.6 \pm 6.65M	
		0.5	17.8 \pm 7.8ib	
	70% EtOH	0.125	7.3 \pm .325t	1.56 tOHory
		0.25	12.1 \pm 2.1 t	
		0.5	16.0 tOHory	

Each values is expressed as mean \pm standard deviation ($n = 3$), * $p < 0.05$ compared to ethanol extract of *Camellia* mistletoe.

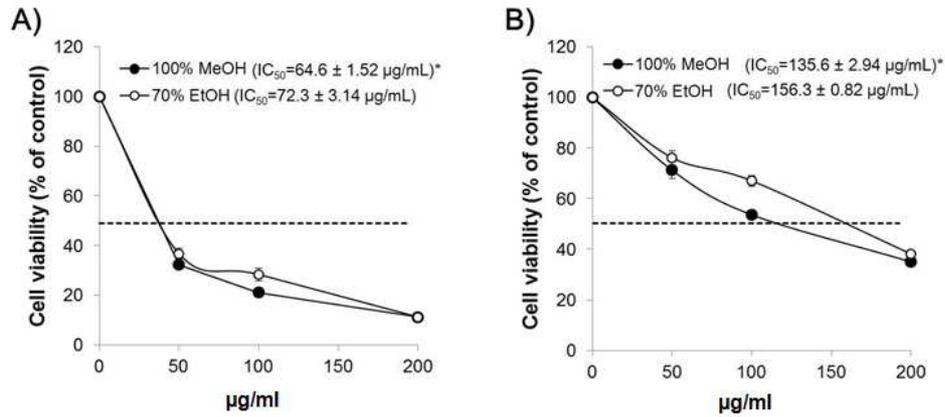


Fig. 1: The percentage of viable HeLa and A375 cells after *Camellia* mistletoe (*K. japonica*) treatment, Cell extracts for 72 h. Each point is the mean±SD of three experiments, * $p < 0.05$ compared to ethanol extract of *Camellia* mistletoe, Cell viability was determined by MTT assay after treatment with 0, 50, 100 and 200 µg/ml of methanol and ethanol

In vitro cytotoxic activity

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 [24]. Medicinal plants are therefore being investigated for their anticancer properties, and the demand for natural anticancer agents increasing.

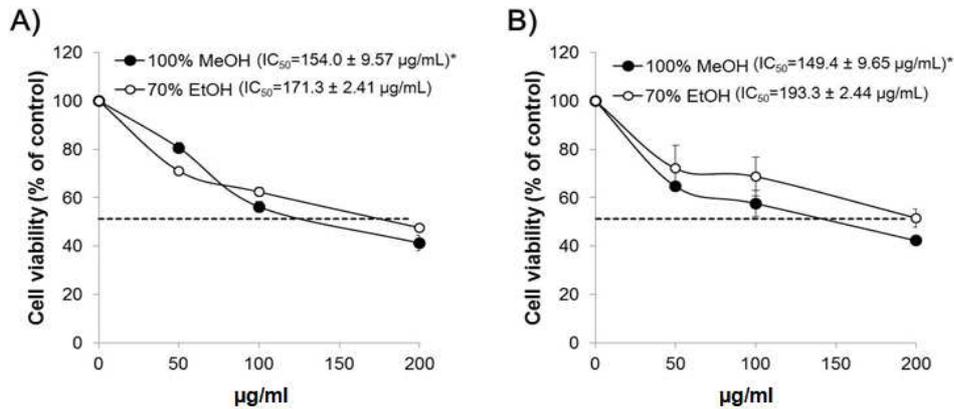


Fig. 2: The percentage of viable HCT116 and HepG2 cells after *Camellia* mistletoe (*K. japonica*) treatment, Cell viability was determined by MTT assay after treatment with 0, 50, 100 and 200 µg/ml of methanol and ethanol extracts for 72h, Each point is the mean±SD of three experiments, * $p < 0.05$ compared to ethanol extract of *Camellia* mistletoe

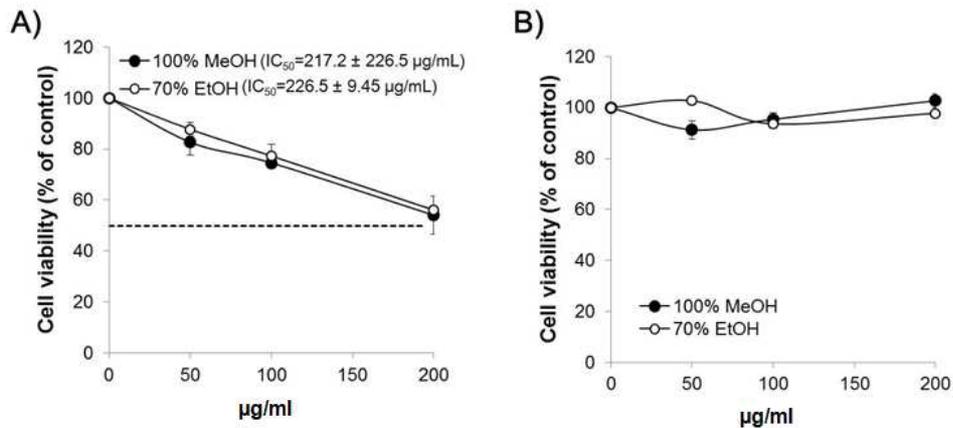


Fig. 3: The percentage of viable A549 and TK6 cells after *Camellia* mistletoe (*K. japonica*) treatment, Cell viability was determined by MTT assay after treatment with 0, 50, 100 and 200 µg/ml of methanol and ethanol extracts for 72 h, Each point is the mean±SD of three experiments, * $p < 0.05$ compared to ethanol extract of *Camellia* mistletoe

In the present study, human cervical cancer (Hela), malignant melanoma (A375), colon cancer (HCT 116), liver cancer (HepG2) and non-small cell lung adenocarcinoma (A549) cells were treated with various concentrations (0-200 $\mu\text{g/ml}$) of *Camellia* mistletoe extracts for 72 h, and then analyzed by MTT assay for the cell viability (% control), as shown in fig. 1-3. Overall, both methanol and ethanol extracts of *Camellia* mistletoe were found to exhibit significantly growth inhibitory effect in all human cancer cell lines, and methanol extract was more active than ethanol extract ($p < 0.05$) (fig. 1-3). The highest cytotoxic activity (IC_{50} of 64.6 $\mu\text{g/ml}$) was obtained against the Hela cell line by the methanol extract of *Camellia* mistletoe (fig. 1), and the rank order of potency of the methanol and ethanol extracts of *Camellia* mistletoe for cell line was A375 (IC_{50} of 135.6 and 156.3 $\mu\text{g/ml}$), HCT116 (IC_{50} of 154.0 and 171.3 $\mu\text{g/ml}$), HepG2 (IC_{50} of 149.4 and 193.3 $\mu\text{g/ml}$), A549 (IC_{50} of 217.2 and 226.5 $\mu\text{g/ml}$), respectively (fig. 1-3). Based on the results obtained, *Camellia* mistletoe extracts were effective in inhibiting all tested cancer cell proliferation. 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 (fig.3B). 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

Our study proposed *Camellia* mistletoe as a candidate for natural lipase inhibitor and anticancer agent. These *in vitro* antilipase and antiproliferative activities of *Camellia* mistletoe extracts seems to be closely correlated with its lectin contents or phenolic components. Further study is also needed to investigate the mechanism of antilipase and antiproliferative activities of *Camellia* mistletoe extracts.

AUTHORS CONTRIBUTION

Design, experimental part of the work and writing of the manuscript was done by the corresponding author Dr. M. Y. Kim.

CONFLICT OF INTERESTS

We declare that there is no conflict of interest

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