

## ORIGINAL ARTICLE

# Selective cytotoxic effect of *Rhododendron luteum* extract on human colon and liver cancer cells

Selim Demir<sup>1</sup>, Ibrahim Turan<sup>2,3</sup>, Yuksel Aliyazicioglu<sup>4</sup>

<sup>1</sup>Department of Nutrition and Dietetics, Faculty of Health Sciences, Karadeniz Technical University, 61080, Trabzon;

<sup>2</sup>Department of Genetic and Bioengineering, Faculty of Engineering and Natural Sciences, Gumushane University,

29100, Gumushane; <sup>3</sup>Medicinal Plants, Traditional Medicine Practice and Research Center, Gumushane University,

29100, Gumushane; <sup>4</sup>Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Turkey

## Summary

**Purpose:** The genus *Rhododendron* is distributed entirely in the world with the exception of South and Central America and Africa, growing in a large diversity of climatic conditions. This genus is a rich source of phenolic compounds, especially flavonoids, essential oils, chromones, terpenoids, and steroids. It has many biological properties such as antioxidant, anti-inflammatory, antiviral, antibacterial, anti-cancer, antidiabetic, immunomodulatory, cardioprotective and hepatoprotective among others due to their polyphenolic constituents. The objective of the current study was to evaluate the antioxidant properties and cytotoxic activity of dimethyl sulfoxide extract of flowers of *Rhododendron luteum* (DEFER) for the first time.

**Methods:** The total polyphenolic contents (TPC), total flavonoid contents (TFC) and ferric reducing antioxidant power (FRAP) of the extract were evaluated using spectrophotometric procedures. The cytotoxic activity of the ex-

tract on three cancers (human breast, colon and liver carcinoma) and human foreskin fibroblast cells was determined using the MTT assay.

**Results:** TPC and FRAP values were found  $54.2 \pm 0.38$  mg gallic acid equivalents and  $164.2 \pm 1.77$  mg trolox equivalents per to g sample, respectively. *R.luteum* extract exhibited selective cytotoxicity against colon and liver cancer cells compared to normal fibroblast cells, while this selective cytotoxicity was not observed in breast cancer cells.

**Conclusion:** Our results demonstrate that the *Rhododendron luteum* may be a great source of antioxidant and antitumor natural agents due to their capability of decreasing cancer cells proliferation.

**Key words:** antioxidant activity, cancer cell lines, cytotoxic effect, polyphenols, *Rhododendron luteum*

## Introduction

The genus *Rhododendron* (Greek rhodon=rose and dendron=tree) is distributed widely around the world with the exception of South and Central America and Africa, growing in a large diversity of climatic conditions [1,2]. The first written record of the species dates back to 401 B.C. and concerned the toxicity of *Rhododendron* honey [3]. The genus *Rhododendron* pertains to the Ericaceae family of plants and includes more than 1000 botanically identified species and approximately 30000 different cultivars. Thus, it constitutes the plant genus

with one of the highest species' variety. *Rhododendrons* are best known for their use in gardens as decoration plants [1,2,4]. Five *Rhododendron* species are determined to grow naturally in Turkey and especially in East Black Sea Region, namely *R.luteum*, *R.caucasicum*, *R.ponticum*, *R.smirnovii*, and *R.ungerni*. These 5 species are non-evergreen short trees with green leaves and have flowers of different colors and an esthetically important role in landscape [5,6]. *R.luteum*, also locally known as "zifin, sifin, cifin, sari ormangulu, egri cicegi or

sari agu”, is found in Trabzon, Turkey [7]. This genus is a source of phenolic compounds, especially flavonoids, tannins, essential oils, chromones (isomers of coumarins), terpenoids, and steroids [1,2]. Up to now, more than 65 flavonoids have been determined in *Rhododendron* species both as aglycones and glycosides. Kaempferol, quercetin, quercitrin, hyperoside, farrerol and polys-tachoside were detected in the leaves of 166 *Rhododendrons* [3,8]. Anthocyanidins and flavonols, such as cyanidin, peonidin and azaleatin, have been found in the flowers of *Rhododendrons* [3]. The medical use of *Rhododendron* genus is limited because of the occurrence of phytotoxins called “grayanotoxins”, a class of terpenoids present in this genus [1,9-11]. Nevertheless, some species of this genus, including low levels of grayanotoxins, are used for medicinal purposes in Turkish and Chinese traditional medicine to treat acute and chronic bronchitis, rheumatism, arthritis, asthma, pain, inflammation, hypertension, and muscle and metabolic diseases [1,6,12]. This genus has many biological properties, such as antioxidant, anti-inflammatory, antiviral, antinociceptive, antibacterial, antifungal, anticancer, antidiabetic, spasmolytic, sedative, immunomodulatory, cardioprotective and hepatoprotective, due to their polyphenolic constituents [1,3,6,13-16]. Polyphenols are a major class of secondary herbal metabolites. It is known that they are good antioxidant molecules due to their high reactivity as hydrogen or electron donors and also to their capability to chelate metal ions [17]. Polyphenols provide protection against reactive oxygen and nitrogen species and have been known to reduce the risk of certain diseases associated with oxidative stress (cancer, coronary heart disease, cardiovascular disease, stroke, etc) [18].

Cancer is a big health problem all over the world and the second leading cause of death after heart diseases [19]. It is a pathological condition where the normal mechanisms of cell cycle regulation are dysfunctioning, either due to excessive cell proliferation, insufficient apoptosis or both [20]. Nowadays, used chemotherapeutic drugs may not be highly effective against some cancer cells and their efficiency may be decreased due to the development of drug resistance [21]. Researchers, therefore, focus on the potential use of natural compounds as chemotherapeutics or complementary agents for the treatment of cancer due to the moderate or low efficiency of the current drugs [22].

Although limited, there is information about

the cytotoxic activity of different *Rhododendron* species [3] and, despite grayanotoxin being among the contents of the *R.luteum*, there is no report about the cytotoxic activity and selectivity of extracts of *R. luteum*. Therefore, we aimed to evaluate the possible antioxidant properties and cytotoxic activities of dimethyl sulfoxide (DMSO) extracts of the flowers of *R.luteum* on human liver, breast and colon cancer cell lines.

## Methods

### Chemicals

DMSO, sodium carbonate, folin reagent, gallic acid, ethanol, aluminum nitrate, potassium acetate, (+)-Catechin, quercetin,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ , potassium ferricyanide, trichloroacetic acid, iron(III) chloride, trolox, cisplatin, trypan blue, thiazolyl blue tetrazolium bromide (MTT dye) were purchased from Sigma (St. Louis, MO, USA). Penicillin-streptomycin and trypsin from Gibco (Paisley, England), Eagle's Minimum Essential Medium (EMEM) from Lonza (Verviers, Belgium), fetal bovine serum (FBS) from Biochrom (Berlin, Germany), and phosphate buffer saline (PBS) tablets from Medicago (Uppsala, Sweden).

### Sample collection

The flowers of *R.luteum* were collected from a rural area of Eastern Black Sea region (Caykara town, Trabzon, Turkey). After identification, the samples were cleaned with tap water and were air-dried at room temperature for 20 days and powdered using blender and milling into fine powder. The powder of fruits were stored and packed in freezer bags at  $-20^\circ\text{C}$  until tested.

### Preparation of extract

The powder of flowers (1 g) was extracted with 20 mL DMSO in a mechanical shaker (Shell Lab, Cornelius, OR, USA) in the dark for 24 hrs at  $45^\circ\text{C}$ . Then it was centrifuged at 4000 rpm for 10 min. The supernatant was first filtered through a medium flow filter paper (Grade 1:11  $\mu\text{M}$  Whatman filter) followed by 0.22  $\mu\text{M}$  syringe filters. Prepared 50000  $\mu\text{g}/\text{mL}$  stock extract was used for the experiments.

### Drug preparation and treatment

Cisplatin was used as a reference anticancer agent for cytotoxicity experiments. It was dissolved in absolute DMSO to prepare 1000  $\mu\text{g}/\text{mL}$  stock solution.

(+)-Catechin was used as a single flavonoid (proanthocyanidin) for cytotoxicity experiments because it is one of the major flavonoids in *Rhododendrons* [1]. It was dissolved in absolute ethanol to prepare 50000  $\mu\text{g}/\text{mL}$  stock solution. External working concentrations of the extract, cisplatin and catechin were prepared by further

dilution with DMSO or ethanol. The final concentration of DMSO and ethanol did not exceed 0.5% in culture media during any experiment, and this concentration did not affect cell morphology or viability.

#### Total polyphenolic content (TPC)

Total polyphenols in the extract were determined using Folin-Ciocalteu reagent, as previously described [23], adapted to microscale. Gallic acid was used as a standard and values were stated as mg gallic acid equivalents (GAE)/g sample.

#### Total flavonoid content (TFC)

Total flavonoids in the extract were determined using aluminum nitrate colorimetric method [24] adapted to microscale. Quercetin was used as a standard and values were stated as mg of quercetin equivalents (QE)/g sample.

#### Ferric reducing antioxidant potential (FRAP)

The ferric reducing power of the extract was determined using the method which is relying on ferric to ferrous ion reduction at low pH [25]. Trolox was used as a standard and values were stated as mg of trolox equivalents (TE)/g sample.

#### Cell culture

Human hepatocellular carcinoma (HepG2), colon adenocarcinoma (WiDr), and breast adenocarcinoma (MCF-7) cancer cell lines and human normal foreskin fibroblast cells were purchased from the American Type Culture Collection (Manassas, VA). All cells were cultured in EMEM supplemented with 2 mM L-glutamine, 10% heat inactivated FBS, 1% penicillin and streptomycin in T-75 flasks, with 5% CO<sub>2</sub> supply at 37°C.

#### Cytotoxicity experiment

The cytotoxic effects of *R.luteum* extract, (+)-Catechin, and cisplatin on 3 human cancer and one human normal cell lines were determined by MTT assay 72 hrs after treatment [26]. Briefly, cells were seeded into a flat-bottomed 96-well cell culture plate at a density of 5000 cells per well. After 24 hrs, mediums were removed and cells were treated with different concentrations of *R.luteum* extract (0-500 µg/mL), (+)-Catechin (0-500 µg/mL) and cisplatin (0-10 µg/mL) for 72 hrs in triplicate. Next, 10 µL of MTT solution (final concentration 0.25 mg/mL) was added to each well and the composed crystals were dissolved in DMSO. Finally, absorbance was measured at 570 nm using a microplate reader (Versamax, Molecular Devices, Sunnyvale, CA). Optical densities (OD) were used to determine the percent of cell viability using the formula [(OD of treated group/OD of control group) × 100] [27]. A log-concentrations vs % cell viability graph were plotted, and IC<sub>50</sub>

values were determined using this logarithmic graph. IC<sub>50</sub> represents the concentration in µg/mL required for 50% inhibition of cell growth compared to negative control cells.

#### Statistics

All data were obtained from three independent experiments. The values were expressed as mean ± standard deviation (mean±SD). TPC, TFC, FRAP and IC<sub>50</sub> values were calculated using Microsoft Excel software.

## Results

The antioxidant properties of the extract were determined by 3 methods: TPC, TFC and FRAP assays. TPC, TFC and FRAP values of the extract are shown in Table 1.

To investigate the cytotoxic activities of *R.luteum* extract, an in vitro assay was performed using 3 human cancer cell lines (HepG2, WiDr, and MCF-7) and one human normal fibroblast cell line. The results expressed as IC<sub>50</sub> and values are listed in Table 2. Cisplatin was used as a reference chemotherapeutic drug. The IC<sub>50</sub> values demonstrated that *R.luteum* extract exhibited non-selective cytotoxic effect on MCF-7 cells, but it had selective cytotoxic activity against HepG2 and WiDr cells compared with normal fibroblast cells. Interestingly, (+)-Catechin did not exhibit selective cytotoxic effect on these 2 cancer cell lines.

**Table 1.** Antioxidant activities of DMSO extract of *Rhododendron luteum* (N=3)

Antioxidant parameters	Mean±SD
Total polyphenolic content <sup>1</sup>	54.2±0.38
Total flavonoid content <sup>2</sup>	18.5±0.98
Ferric reducing antioxidant power (FRAP) <sup>3</sup>	164.2±1.77

<sup>1</sup>Total phenolic content expressed in mg of gallic acid equivalent (GAE) per gram of dry plant weight.

<sup>2</sup>Total flavonoid content expressed in mg of quercetin equivalent (QE) per gram of dry plant weight.

<sup>3</sup>Expressed as mg trolox equivalents (TE) per gram of dry plant weight.

## Discussion

There has been excellent interest in the antiproliferative properties of natural products, because they are believed to be relatively non-toxic and have been used as traditional medicines for centuries worldwide [28]. Nowadays, over 70% of anticancer agents are derived from natural

**Table 2.** Cytotoxic activity (IC<sub>50</sub>, µg/mL\*) of *R. luteum* extract and other test compounds (N=3)

	Test compounds		
	<i>R. luteum</i> extract Mean±SD	(+)-Catechin Mean±SD	Cisplatin Mean±SD
MCF-7	203.2±5.7	103.8±4.5	0.53±0.04
HepG2	50.2±1.5	243.9±1.8	2.41±0.13
WiDr	42.9±0.5	483.1±5.9	1.13±0.04
Fibroblast	196.5±2.4	185.6±2.8	4.95±0.22

\*IC<sub>50</sub> is defined as the concentration inhibiting 50% of cell growth (viability) after treatment with the *R.luteum* extract for 72 hrs, according to the MTT assay.

products [29]. The genus *Rhododendron*, pertained to the *Ericaceae* family of plants, includes more than 1000 botanically identified species and approximately 30000 different cultivars [1,2,4]. Five *Rhododendron* species are determined to growing naturally in Turkey and especially in East Black Sea Region, namely *R.luteum*, *R.caucasicum*, *R.ponticum*, *R.smirnovii*, and *R.ungerni* [5,6]. These genera are a source of phenolic compounds, essential oils, chromones, terpenoids, and steroids [1,2]. Besides, some species of this genus are used for medicinal purposes in Turkish and Chinese traditional medicinal system [1,6,12].

Mostly before the extraction procedure, plant samples are treated by milling, grinding and homogenization. Several methods have been used for extraction of active components from plant materials. These methods are maceration, ultrasonic (sonication), soxhlet and microwave extraction. For maceration technique, organic solvents are used to dissolve the components in plant materials directly without producing heat, so this technique is suitable for heat labile and heat stable substances. Many solvents (water, ethanol, methanol, ethyl acetate, dimethyl sulfoxide, hexane or acetone) are used to preparing plant extracts [30]. For this reason, we have preferred preparing DMSO extracts of flowers of *R.luteum* with the maceration technique.

The determination of TPC and TFC are important in various natural products. These physico-chemical methods are frequently used for evaluating the antioxidant capacities of plant samples since they are useful, rapid and cheap assays [30]. A direct relationship has been found between the total polyphenolic contents and the antioxidant capacity of many fruits and vegetables [31]. In our study TPC and TFC values of the extract were found 54.2±0.38 mg GAE and 18.5±0.98 mg

QE per to g sample, respectively (Table 1). Total polyphenolic contents in methanolic extracts of the leaves of *Rhododendron* genus were changed between 37.3 and 319 mg GAE/g sample [2,12], while the total flavonoid content values in methanolic extracts of leaves of *Rhododendron* genus were found 11.5 to 137.1 mg QE/g sample [2] in the literature. In our study FRAP values of the extract were found 164.2±1.77 mg TE/g sample (Table 1), while FRAP values of methanolic extracts of leaves of *Rhododendron* genus were found 225-384 Catechin Equi/g sample [12] and 0.16-2.03 ascorbic acid Equi/g sample [14]. The antioxidant activity results of the present study are thus largely in agreement with those of other studies [2,12,14]. Small differences may have arisen from the plant species, sampling part of the plant, type of extraction method, geographic region and post-harvesting conditions.

Few studies have reported about the anti-proliferative activity of *Rhododendron* genus and some isolated bioactive compounds from them [3,32-36]. The effectiveness of anticancer therapy is evaluated by the ability to initiate apoptosis or cell cycle arrest in cancer cells. Apoptosis induction and cell cycle arrest are recommended as main mechanisms for the anticancer activities of natural products [22,37]. Way et al. demonstrated that different extracts of *Rhododendron formosanum* and its chemical constituents present antiproliferative effects on non-small-cell lung carcinoma cells by stimulating apoptosis [32], while Seephonkai et al. have shown that three isolated compounds (Ferruginenes A-C) from *Rhododendron ferrugineum* have cytotoxic activity against HL-60, HeLa-S3, and MCF-7 cancer cell lines [33]. In another study, Li et al. found that purified hyperin from *Manchurian Rhododendron* leaves has anticancer activity against human en-

ometrial cancer cell line (RL952) by inducing apoptosis via Ca<sup>2+</sup>-related mitochondrial apoptotic pathway [34], while Byun et al. shown that the 70% ethanolic extract of the leaves of *Rhododendron brachycarpum* exhibited anticancer activity against some human cancer cell lines (A549, AGS, Hep3B, and MCF-7) for the dose of 1 mg/mL [36]. Recently, Way et al. reported that Cinnamtannin D1 from *Rhododendron Formosanum* induces autophagy in lung cancer cells through inhibition of Akt/mTOR and activation of ERK1/2 pathways [38].

Selectivity (no toxic effects on healthy cells) and effectiveness (high efficacy against multiple cancers) are the desired two main properties from an effective and acceptable anticancer agent [39]. For this reason in the present study we demonstrated the cytotoxic effects of *R.luteum* extract against three human tumor and one human normal cell lines. In the present study, *R.luteum* extract exhibited non-selective cytotoxic effect on MCF-7 cells, but it had a moderate selective cytotoxic effect against HepG2 and WiDr cells compared with normal fibroblast cells. Our data showed that IC<sub>50</sub> values of the extract (belonging to HepG2 and

WiDr cells) were lower than IC<sub>50</sub> values of (+)-Catechin and much higher of cisplatin (Table 2). The selective cytotoxic effect of *R.luteum* extract may therefore be attributed to a synergism between polyphenolic compounds and other constituents in the flower rather than to the effect of a specific phytochemical or a class of phytochemicals.

It is known that the Ericaceae family is a rich resource of polyphenolic compounds, such as caffeoylquinic acids, catechin, quercetin, myricetin, taxifolin, hyperoside, quercitrin and their derivatives [1,3,6,40].

This study is the first to report about the cytotoxic activity of *R.luteum* extract on cancer cells and our results demonstrated that *R.luteum* might be an novel and promising therapeutic agent for cancer treatment in clinical practice. However, further investigations are needed to confirm *in vivo* effects and also to clarify the molecular mechanism(s) of the cytotoxic effects and the study of individual constituents from *R.luteum*.

## Conflict of interests

The authors declare no conflict of interests.

## References

- Jaiswal R, Jayasinghe L, Kuhnert N. Identification and characterization of proanthocyanidins of 16 members of the Rhododendron genus (Ericaceae) by tandem LC-MS. *J Mass Spectrom* 2012;47:502-515.
- Prakash D, Upadhyay G, Singh BN, Dhakarey R, Kumar S, Singh KK. Free-radical scavenging activities of Himalayan Rhododendrons. *Current Sci* 2007;92:526-532.
- Popescu R, Kopp B. The genus Rhododendron: An ethnopharmacological and toxicological review. *J Ethnopharmacol* 2013;147:42-62.
- Sawidis T, Theodoridou T, Weryszko-Chmielewska E, Bosabalidis AM. Structural features of Rhododendron luteum flower. *Biologia* 2011;66:610-617.
- Usta A, Yayli B, Kahrinman N, Alpaya Karaoğlu S, Yayli N. Composition and antimicrobial activity of essential oil from the flower of Rhododendron luteum sweet. *Asian J Chem* 2012;24:1927-1930.
- Erdemoglu N, Akkol EK, Yesilada E, Calis I. Bioassay-guided isolation of anti-inflammatory and antinociceptive principles from a folk remedy Rhododendron ponticum L. leaves. *J Ethnopharmacol* 2008;119:172-178.
- Alan S, Kurkcuglu M, Goger F, Canbaser KH. Morphological, chemical and indumentum characteristics of Rhododendron luteum sweet (Ericaceae). *Pak J Bot* 2010;42:3729-3737.
- Wang Q, Zhang B, Yu JL. Ferrerol inhibits IL-6 and IL-8 production in LPS-stimulated human gingival fibroblasts by suppressing PI3K/AKT/NF-κB signaling pathway. *Arch Oral Biol* 2016;62:28-32.
- Zhang WD, Jin HZ, Chen G et al. A new grayanane diterpenoid from Rhododendron decorum. *Fitoterapia* 2008;79:602-604.
- Gunduz A, Turedi S, Uzun H, Topbas M. Mad honey poisoning. *Am J Emerg Med* 2006;24:595-598.
- Carballeira NM, Cartagena M, Tasdemir D. Fatty acid composition of Turkish Rhododendron species. *J Am Oil Chem Soc* 2008;85:605-611.
- Lin CY, Lin LC, Ho ST, Tung YT, Tseng YH, Wu JH. Antioxidant activities and phytochemicals of leaf extracts from 10 native Rhododendron species in Taiwan. *Evid Based Complem Altern Med* 2014;Article ID 283938.
- Lee SH, Sancheti SA, Bafna MR, Sancheti SS, Seo SY. Acetylcholinesterase inhibitory and antioxidant properties of Rhododendron yedoense var. poukhanense bark. *J Med Plants Res* 2011;5:248-254.
- Prakash T, Fadadu SD, Sharma UR et al. Hepatoprotective activity of leaves of Rhododendron arboreum in CCl<sub>4</sub> induced hepatotoxicity in rats. *J Med Plants Res* 2008;2:315-320.
- Orhan I, Sener B, Choudhary MI, Khalid A. Acetyl-

- cholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. *J Ethnopharmacol* 2004;91:57-60.
16. Erturk O, Karakas FP, Pehlivan D, Nas N. The antibacterial and antifungal effects of *Rhododendron* derived mad honey and extracts of four *Rhododendron* species. *Turk J Biol* 2009;33:151-158.
  17. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci* 1997;2:152-159.
  18. Androustopoulos VP, Papakyriakou A, Vourloumis D, Tsatsakis AM, Spandidos DA. Dietary flavonoids in cancer therapy and prevention: Substrates and inhibitors of cytochrome P450 CYP1 enzymes. *Pharmacol Ther* 2010;126:9-20.
  19. Reddy L, Odhav B, Bhoola KD. Natural products for cancer prevention: a global perspective. *Pharmacol Ther* 2003;99:1-13.
  20. Prakash O, Kumar A, Kumar P. Anticancer potential of plants and natural products: a review. *AJPS* 2013;1:104-115.
  21. Gottesman MM. Mechanism of cancer drug resistance. *Annu Rev Med* 2002;53:615-627.
  22. Nobili S, Lippi D, Witort E et al. Natural compounds for cancer treatment and prevention. *Pharmacol Res* 2009;59:365-378.
  23. Slinkard K, Singleton VL. Total phenol analyses: automation and comparison with manual methods. *Am J Enol Viticult* 1977;28:49-55.
  24. Mohammadzadeh S, Sharriatpanahi M, Hamed M, Amanzadeh Y, Ebrahimi SES, Ostad SN. Antioxidant power of Iranian propolis extract. *Food Chem* 2007;103:729-733.
  25. Benzie IFF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method Enzymol* 1999;299:15-27.
  26. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.
  27. Xuan H, Li Z, Yan H et al. Antitumor activity of Chinese propolis in human breast cancer MCF-7 and MDA-MB-231 cells. *Evid Based Compl Alternat Med* 2014; Article ID 280120.
  28. Fauzi AN, Norazmi MN, Yaacob NS. Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. *Food Chem Toxicol* 2011;49:871-881.
  29. Karikas GA. Anticancer and chemopreventing natural products: Some biochemical and therapeutic aspects. *J BUON* 2010;15:627-638.
  30. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 2010;15:7313-7352.
  31. Vinson JA, Su X, Zubik L, Bose P. Phenol antioxidant quality in food: fruits. *J Agric Food Chem* 2001;49:5315-5321.
  32. Way TD, Tsai SJ, Wang CM, Ho CT, Chou CH. Chemical constituents of *Rhododendron formosanum* show pronounced growth inhibitory effect on non-small-cell lung carcinoma cells. *J Agric Food Chem* 2014;62:875-884.
  33. Seephonkai P, Popescu R, Zehl M, Krupitza G, Urban E, Kopp B. Ferruginenes A-C from *Rhododendron ferrugineum* and their cytotoxic evaluation. *J Nat Prod* 2011;74:712-717.
  34. Li FR, Yu FX, Yao ST, Si YH, Zhang W, Gao LL. Hyperin extracted from Manchurian *rhododendron* leaf induces apoptosis in human endometrial cancer cells through a mitochondrial pathway. *Asian Pac J Cancer Prev* 2012;13:3653-3656.
  35. ManiKumar P, Pardhu G, Ravinder S, Lakshmi V, Tejaswini K, Vishwanath PB. Phytochemical screening, antimicrobial, antiproliferative properties of *Rhododendron ponticum* on prostate epithelial cancer cells. *J Pharm Sci Res* 2011;3:1552-1555.
  36. Byun KS, Lee YW, Jin HJ et al. Genotoxicity and cytotoxicity in human cancer and normal cell lines of the extracts of *Rhododendron brachycarpum* D Don leaves. *Korean J Med Crop Sci* 2008;13:199-205.
  37. Fulda S. Modulation of apoptosis by natural products for cancer therapy. *Planta Med* 2010;76:1075-1079.
  38. Way TD, Tsai SJ, Wang CM, Jhan YL, Ho CT, Chou CH. Cinnamtannin D1 from *Rhododendron formosanum* induces autophagy via the inhibition of Akt/mTOR and activation of ERK1/2 in non-small cell lung carcinoma cells. *J Agric Food Chem* 2015;63:10407-10417.
  39. Galati G, O'Brien PJ. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Radic Biol Med* 2004;37:287-303.
  40. Qiang Y, Zhou B, Gao K. Chemical constituents of plants from the genus *Rhododendron*. *Chem Biodivers* 2011;8:792-815.