Research Paper

In vitro Screening for Antioxidant, Antimicrobial, and Antidiabetic Properties of Some Korean Native Plants on Mt. Halla, Jeju Island

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In this study, *Prunus padus*, *Lonicera caerulea*, *Berberis amurensis*, and *Ribes maximowiczianum*, which are mainly distributed on Mt. Halla, Jeju Island, have been investigated for their antioxidant, antimicrobial, and antidiabetic activities. The methanol extracts of *R. maximowiczianum* leaves and *P. padus* branches exhibited significant and dose-dependent antioxidant activity including electron-donation ability and reducing power. To analyze the antimicrobial activity, each extract was tested by a serial two-fold dilution method against five selected gram-positive bacteria and four gram-negative bacteria, and this suggested that *P. padus* branches possessed the maximum antimicrobial activity against most of the gram-positive bacteria tested. In addition, the methanol extracts of *P. padus* branches exhibited the highest α-glucosidase inhibitory activity with an IC_{50} value of 1.0±0.1 µg/ml, indicating that *P. padus* is a promising source as a herbal medicine.

Key words: Antioxidant activity, antimicrobial activity, *α-glucosidase inhibitory effect, Prunus padus, Lonicera caerulea, Berberis amurensis, Ribes maximowiczianum*

Natural products, mostly from plants, have begun to gain worldwide interest for promoting healthcare, and have been used as conventional or complementary medicines due to toxicity and side effects of synthetic drugs[1]. In addition, natural products are known not only as a rich source of structurally diverse substances with a wide range of biological activities, but also as a primary source for synthesized drugs[1,2]. Therefore, the investigation of the pharmaceutical properties of medicinal plants and the analyses of their natural products are an important aspect when developing alternative or adjunctive therapies. The biological and pharmaceutical activities in medicinal plants are mostly mediated by the presence of secondary metabolites including phenolic and flavonoid compounds. These compounds exhibit a wide range of pharmaceutical properties, such as antioxidant, antimicrobial, antiinflammatory and anticancer properties[3]. Although a variety of plants are known to be good sources of these compounds, their contents are dependent on a number of factors including the climatic conditions, ripeness of the material, their tissues, and genetic factors[4].

Jeju Island in South Korea is known as a crossroads for several migration routes[5]. In addition, due to its geographical position, elevation and topography, the vertical distribution of temperatures and pressures from a subtropical to a subarctic zone allow for the generation of a unique ecosystem on Jeju Island[6,7]. So far, over 1,990 species including 719 species of edible plants have been classified[8,9]. This indicates that Jeju Island has a rich diversity of plants. *Prunus padus* L., *Berberis amurensis var. quelpaertensis* Nakai, *Lonicera caerulea* L., and *Ribes maximowiczianum* Kom., which are

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mainly distributed on Mt. Halla, Jeju Island, have been widely used as a traditional medicine, with beneficial effects in numerous diseases such as stroke, neuralgia, hepatitis, diarrhea and ocular disorder[10-12]. The phytochemical analysis revealed that *P. padus*, *L. caerulea* are a rich source of anthocyanins and flavonoids, which display potential health-promoting effects[11,13]. In addition, berberine (isoquinoline alkaloid), which possesses various biological activities including antimalarial, anticancer and antiAlzheimer disease, has been isolated from the roots and stem bark of *Berberis* species[12]. These indicate that these plants might be potential resources as a crude drug and dietary health supplements, although there are only a few systematic studies regarding their pharmaceutical potentials. Therefore, in this study, leaves and branches of *P. padus* (fig. 1a), *L. caerulea* (fig. 1b), *B. amurensis* (fig. 1c), and *R. maximowiczianum* (fig. 1d) have been investigated for their antioxidant, antimicrobial, and antidiabetic activities. Methanol extracts from branches of *P. padus* exhibited a high level of radical scavenging activity, reducing power, and α-glucosidase-inhibition activity compared with other extracts. These findings suggest that *P. padus* is a new potential source for herbal medicine.

MATERIALS AND METHODS

Branches and leaves of *P. padus*, *L. caerulea*, *B. amurensis*, and *R. maximowiczianum* were obtained from the Research Institute for Hallasan (Jeju). The ground materials were soaked with methanol for 24 h, and were sonicated at 55° in an ultrasonic bath (Power sonic 520, Hwashin co., Korea). After filtration, each extract was evaporated using a rotary vacuum evaporator.

Analysis of total phenolic and flavonoid content:
The total phenolic contents were determined with Folin-Ciocalteu reagent by using gallic acid as a standard phenolic compound. Each extract (0.1 ml) was mixed with 50 µl of 2 N Folin-Ciocalteu reagent. After 5 min, the mixture was mixed with 0.3 ml of 20% sodium carbonate, and incubated for 15 min at room temperature. Then, 1 ml of distilled water was added to the mixture. The absorbance was measured at 725 nm using a UV-spectrophotometer (UV-1800, Simadzu, Tokyo, Japan). The level of the total phenolic compounds in each extract was calculated as µg of gallic acid equivalents per gram of extract using the equation obtained from the standard gallic acid graph.

To analyze the total flavonoid content in each extract, 0.5 ml of each extract was added to test tubes containing 0.1 ml of 10% aluminum nitrate (w/v), 0.1 ml of 1 M potassium acetate, and 4.3 ml of 80% ethanol. After 40 min incubation at room temperature, the absorbance was determined at 415 nm. The total flavonoid content was determined in micrograms of quercetin equivalents (QE) per gram of extract.

Analysis of electron-donation ability:
Based on the 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity, the EDA of each extract was determined. The different concentrations of each extract in 4 ml methanol were mixed with 1 ml DPPH (0.15 mM in MeOH). The mixture was incubated for 30 min at room temperature, and then the absorbance was measured at 517 nm using a UV/Vis spectrophotometer. The EDA was calculated as a reduction rate in the absorbance by using the following equation: EDA (%)=$[1-(A_1/A_0)]\times100$, where A_1 is the absorbance value of the sample,

Fig. 1: Pictorial image of plants used in study.
General view of *Prunus padus* L. (a), *Lonicera caerulea* L. (b), *Berberis amurensis* var. *quelpaertensis* Nakai (c), and *Ribes maximowiczianum* Kom. (d).

and A_0 is the absorbance value of the control. The means and standard errors were calculated from three independent experiments.

Determination of the total reduction capability via Fe^{3+}-Fe^{2+} transformation:

To analyze the total reducing power of all the extracts, different concentrations of extracts (100, 200 and 300 µg/ml) were mixed with 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferricyanide. After incubation at 50° for 20 min, 2.5 ml of 10% trichloroacetic acid was added to the reaction mixture, and then centrifuged at 650 rpm for 10 min. The upper layer (0.5 ml) was mixed with distilled water (0.5 ml) and ferric chloride (0.1 ml, 0.1%). The absorbance was measured at 700 nm using a UV/Vis spectrophotometer.

Determination of antimicrobial activity:

The test strains used for the analysis of antimicrobial activity included five gram-positive bacteria; *Bacillus atrophaeus* (KACC 14742), *Kocuria rhizophila* (KACC 14744), *Micrococcus luteus* (KACC 14819), *Staphylococcus epidermidis* (KACC 14822), and *Bacillus subtilis* subsp. Spizizenii (KACC 14741), and four gram-negative bacteria; *Klebsiella pneumoniae* (KACC 14816), *Enterobacter cloacae* (KACC11958), *Salmonella enterica* subsp. enterica (KACC 10769), *Pseudomonas aeruginosa* (KACC 10186). All strains were obtained from the Korean Agricultural Culture Collection (KACC) in South Korea. The degree of antimicrobial activity was assayed by a serial two-fold dilution method, to determine the minimum inhibitory concentration (MIC) of each extract, as described by Olajuyigbe and Afolayan[14].

α-Glucosidase inhibitory effect:

The α-glucosidase inhibitory effect of each methanol extract was assayed according to the procedure described previously by Kim *et al.*[15], with minor modifications. 50 µl of each extract was mixed with 50 µl α-glucosidase (0.5 U/ml) and 50 µl of 0.2 M potassium phosphate buffer (pH 6.8), and the mixture was incubated at 37° for 15 min. 3 mM of the substrate (*p*-nitrophenyl glucopyranoside; *p*NPG) added to the mixture to start the reaction. The reaction mixture was incubated at 37° for 10 min and was stopped by adding 750 µl of 0.1 M Na_2CO_3. The α-glucosidase activity was analyzed by measuring the *p*-nitrophenol released from *p*NPG at 405 nm using a spectrophotometer. The α-glucosidase inhibitory effects of each extract were calculated as: Inhibition rate (%)=[1-(Abs_{sample}–Abs_{blank})/$Abs_{control}$]×100, where Abs_{sample} represents the absorbance of the experimental sample, Abs_{blank} represents the absorbance of the blank, and $Abs_{control}$ represents the absorbance of the control.

Statistical analysis:

Data were subjected to an analysis of variance (ANOVA), and the means were compared via Duncan's multiple range tests at $P<0.05$, which were used to determine the significance of the means.

RESULTS AND DISCUSSION

A number of plants are considered to be good sources of phenolic and flavonoid compounds, which have pharmaceutical properties, such as antioxidant and antimicrobial activities[16]. Therefore, plant-derived phenolic and flavonoid compounds have earned considerable interest. To investigate the pharmaceutical properties of *P. padus*, *L. caerulea*, *B. amurensis* and *R. maximowiczianum*, we firstly determined the total phenol (TPC) and flavonoid content (TFC) from methanol extracts of these plants. The highest level of total phenolic compounds (2956±152 µg GAE/g) was found in *R. maximowiczianum* leaf extract (RmL), whereas *B. amurensis* branch extract (BaB) contained the lowest level of TPC (986±6 µg GAE/g) (Table 1). In the case of TFC, the extract of *L. coerulea* leaves (LcL) and RmL exhibited a higher level of TFC compared with other extracts. In addition, the branch extracts of *P. padus*

TABLE 1: TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT OF METHANOL EXTRACTS FROM KOREAN NATIVE PLANTS ON MT. HALLA

Plants	Parts	Remark	Total phenolic (µg GAE/g)*	Total flavonoid (µg QE/g)#
Prunus padus L.	Branch	PpB	1735±43^{b}	32.3±1.4^{g}
	Leaves	PpL	1180±49^{d}	210.8±2.6^{c}
Lonicera caerulea L.	Branch	LcB	1310±14^{c}	96.5±5.3^{e}
	Leaves	LcL	1074±49^{e}	371.8±2.6^{a}
Berberis amurensis var. quelpaertensis Nakai	Branch	BaB	986±6^{f}	36.6±0.0^{f}
	Leaves	BaL	1110±25de	165.8±2.6^{d}
Ribes maximowiczianum Kom.	Branch	RmB	1026±55^{ef}	5.2±0.0^{h}
	Leaves	RmL	2956±152^{a}	339.1±5.3^{b}

*Total phenolic content analyzed as GAE µg/g of extract; values are the mean±SD of triplicates analysis, #Total flavonoid content analyzed as QE µg/g of extract; values are the mean±SD of triplicates analysis. Each value represents the mean±SD, and the means were significantly different as calculated from a paired Duncan's test at $P<0.05$. a-f means followed with the same letters in each column are not significantly different according to Duncan's multiple range test ($P<0.05$). SD: Standard deviation, GAE: gallic acid equivalent, QE: quercetin equivalent

(PpB, 1735±43 μg GAE/g) and *L. caerulea* (LcB, 1310±14 μg GAE/g) contained higher levels of TPC compared with their leaf extracts, PpL (1180±49 μg GAE/g) and LcL (1074±49 μg GAE/g), respectively, whereas higher levels of TFC were found in PpL (210.8±2.6 μg QUE/g) and LcL (371.8±2.6 μg QUE/g) compared with PpB (32.3±1.4 μg QUE/g) and LcB (96.5±5.3 μg QUE/g) (Table 1). In addition, the extracts from the leaves of *R. maximowiczianum* (RmL) and *B. amurensis* (BaL) showed higher levels of TPC and TFC than their branch extracts. Although these differences might be due to genetic, seasonal, and agronomic factors, these findings indicate that the leaves of *R. maximowiczianum* are a potential rich source of phenolic and flavonoid compounds.

Free radicals produced by several redox reactions in the human body are implicated in contributing to protein oxidation, DNA damage, cancer, immunosuppression, inflammation, diabetes and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases[17-20]. The natural compounds from plants such as vitamins (ascorbate, tocopherol, and cartenoids), polyphenolics and flavonoids play an important role in the defense against free radicals. Consequently, they have been used as supplementary antioxidants[1,21]. The presence of high levels of TPC and TFC in RmL indicates that the leaves of *R. maximowiczianum* might be a good source for an antioxidant agent. To investigate this antioxidant activity, we analyzed the EDA of methanol extracts from the selected plants using DPPH method. As shown in Table 2, RmL displayed the highest antioxidant activity (RC_{50}=33.8±0.2 μg/ml) compared with other extracts, supporting the idea that the polyphenolic constituents are responsible for the EDA of *R. maximowiczianum*. A similar phenomenon has been observed in the extract of *P. padus* leaves and branches. However, LcL exhibited a higher EDA compared to LcB (Table 2), although LcL contained a lower level of TPC than LcB (Table 1). This might be due to the presence of a higher level of TFC in LcL, and suggests that flavonoids are major players as free radical scavengers in *L. coerulea*.

To further characterize the antioxidant properties in the extracts, we investigated Fe^{3+}-Fe^{2+} transformation in the presence of each extract. As we expected, RmL exhibited the highest reductive capacity compared to other extracts (Table 3). RmL (100 μg/ml) exhibited a 0.74±0.03 (OD_{700} value), while 300 μg/ml RmB

TABLE 2: ELECTRON DONATION ABILITY OF METHANOL EXTRACTS FROM KOREAN NATIVE PLANTS ON MT. HALLA

Plant	Parts	Remark	Concentration (μg/mL)	EDA (%)
Prunus padus L.	Branch	PpB	50	62.82±1.76^{b}
			100	85.82±4.42^{b}
	Leaves	PpL	50	34.51±0.23^{c}
			100	66.86±3.51^{d}
Lonicera caerulea L.	Branch	LcB	50	22.30±1.57^{e}
			100	31.22±0.97^{h}
	Leaves	LcL	50	33.44±1.38^{c}
			100	69.10±0.74^{c}
Berberis amurensis var. quelpaertensis Nakai	Branch	BaB	50	23.08±1.12^{de}
			100	37.16±0.24^{g}
	Leaves	BaL	50	11.69±0.87^{f}
			100	13.52±0.67^{i}
Ribes maximowiczianum Kom.	Branch	RmB	50	25.89±0.27^{d}
			100	50.44±0.81^{f}
	Leaves	RmL	50	78.77±0.88^{a}
			100	94.94±0.40^{a}

Each value represents the mean±SD, and the means were significantly different as calculated from a paired Duncan's test at $P<0.05$, a-f means followed with the same letters in each column are not significantly different according to Duncan's multiple range test ($P<0.05$), SD: standard deviation, EDA: electron-donation ability

TABLE 3: REDUCING POWER OF METHANOL EXTRACTS FROM KOREAN NATIVE PLANTS ON MT. HALLA

Plant	Parts	Remark	Concentration (μg/mL)	Abs (700 nm)
Prunus padus L.	Branch	PpB	100	0.51±0.01^{b}
			200	0.74±0.01^{b}
			300	0.99±0.00^{b}
	Leaves	PpL	100	0.34±0.04^{d}
			200	0.62±0.01^{c}
			300	0.88±0.00^{c}
Lonicera caerulea L.	Branch	LcB	100	0.28±0.00^{e}
			200	0.46±0.00^{e}
			300	0.60±0.01^{f}
	Leaves	LcL	100	0.38±0.01^{c}
			200	0.58±0.00^{d}
			300	0.81±0.01^{d}
Berberis amurensis var. quelpaertensis Nakai	Branch	BaB	100	0.26±0.01^{e}
			200	0.46±0.00^{e}
			300	0.63±0.00^{e}
	Leaves	BaL	100	0.12±0.00^{g}
			200	0.21±0.01^{g}
			300	0.30±0.00^{g}
Ribes maximowiczianum Kom.	Branch	RmB	100	0.22±0.00^{f}
			200	0.44±0.00^{f}
			300	0.64±0.01^{e}
	Leaves	RmL	100	0.74±0.03^{a}
			200	1.11±0.02^{a}
			300	1.20±0.00^{a}

Each value represents the mean±SD, and the means were significantly different as calculated from a paired Duncan's test at $P<0.05$, a-f means followed with the same letters in each column are not significantly different according to Duncan's multiple range test ($P<0.05$), SD: standard deviation

(methanol extract of *R. maximowiczianum* branch) displayed 0.64±0.01 (OD_{700} value). This indicates that polyphenolic compounds are the major naturally occurring antioxidants in *R. maximowiczianum*. In the case of *B. amurensis* extract, BaB displayed higher reductive capacity (Table 3), as well as a higher EDA (Table 2) than BaL, although BaB contained a lower level of TPC and TFC compared to BaL (Table 1). One of the possibilities for this non-correlation is that some of the polyphenols, which exist in BaB, are extremely active owing to their structural characteristics even if they are present in smaller quantities[22]. Other possibilities are that non-polyphenol-type compounds such as polysaccharides, which possess strong antioxidant activities[23], might be the active compounds in BaB.

Although a number of new antibiotics have been developed during the last few decades, multiple drug resistance in human pathogenic microorganisms has increased due to indiscriminate use of commercial antibiotics[24]. In addition to this problem, antibiotics are associated with side effects such as immunosuppression, allergic reactions and hypersensitivity[25]. Therefore, alternative antibiotics including plant extracts and phytochemicals are needed to develop treatments for infectious disease. To investigate antimicrobial activity, we analyzed MIC of methanol extracts from the selected plants using the serial two-fold dilution method. PpB and LcB showed antimicrobial activity against most of the gram-positive bacteria tested, whereas none of the extracts, except PpB, exhibited any activity against gram-negative bacteria (Table 4). This might be due to differences in the cell wall composition between gram-negative and gram-positive bacteria. PpB was most active against *Kocuria rhizophila* (MIC=125 µg/ml) in comparison to all the bacteria tested. In addition, LcB showed antimicrobial activity against *Kocuria rhizophila* (MIC=250 µg/ml) and *Bacillus subtilis* (MIC=250 µg/ml). However, leaf extracts of both plants (*P. padus* and *L. coerulea*) contained no or lower antimicrobial activity compared with their branch extracts (Table 4). This suggested that the antimicrobial activity of *P. padus* and *L. coerulea* extracts might be mediated by TPC rather than by the TFC.

World ethnobotanical information indicates that a number of herbal medicines from plants (more than 800 plants) are used for controlling hyperglycemia[26]. In type 2 diabetes mellitus (DM), α-glucosidase inhibitors such as acarbose, 1-deoxynojirimycin and genistein, which are isolated from natural sources, are beneficial in delaying glucose intake with low hypoglycemic effect[27,28]. These suggest the potential and opportunity of medicinal plants to be used for the prevention and management of DM. To identify the potential of *P. padus*, *L. caerulea*, *B. amurensis* and *R. maximowiczianum* as antidiabetic agents, the methanol extracts from each plant were tested for α-glucosidase inhibitory activity. Extracts from *P. padus*, and *L. caerulea* branches significantly inhibited α-glucosidase, whereas LcL showed the lowest inhibitory effect (Table 5). In addition, the leaf extracts and branch extracts of each plant showed different levels of inhibitory effect on α-glucosidase. Interestingly, PpB, LcB, and BaL contained higher levels of TPC and α-glucosidase inhibitory activity than PpL, LcL, and BaB, respectively (Tables 1 and 5). Plant phenolic compounds are known to modulate the enzymatic breakdown of carbohydrate due to their ability to bind with α-glucosidase[29]. Thus, the variation in α-glucosidase inhibitory activity between the organic extracts might be due to the level of phenolic compounds. Based on metabolite profiling of *P. padus* leaves, six phenolic compounds including astragalin and chlorogenic acid have been identified[30]. Astragalin is known as a glycation inhibitor[31] and antioxidant agent[32], and it has an inhibitory effect on α-glucosidase activity[33]. In addition, chlorogenic acid is a highly hydrophilic natural compound containing a catechol group like catechin, which effectively inhibits α-glucosidase and α-amylase activities[34,35]. Although chlorogenic acid, known as an antioxidant agent[36], exhibited a low level of α-glucosidase inhibitory activity, alkyl chlorogenic acid derivatives have been suggested as potential α-glucosidase inhibitors[35]. Taken together, these findings indicate that astragalin and chlorogenic acid (or its derivatives) should be the major bioactive compounds of *P. padus*.

In this study, we analyzed the antioxidant, antimicrobial, and antidiabetic activities of *P. padus*, *L. caerulea*, *B. amurensis*, and *R. maximowiczianum*. The overall results of the present study suggest that the methanol leaf extract of *R. maximowiczianum* could be useful as a source of natural antioxidant agents. In addition, the branch extract of *P. padus* was shown to possess notable pharmaceutical activities,

TABLE 4: ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACTS FROM KOREAN NATIVE PLANTS ON MT. HALLA

Plant	Parts	Remark	MIC (μg/mL)								
			B.a.	K.r.	M.l.	St.e.	B.s.	K.p.	E.c.	S.e.	P.a.
Prunus padus L.	Branch	PpB	250	125	>1000	250	500	1000	500	500	1000
	Leaves	PpL	500	>1000	>1000	>1000	500	>1000	>1000	>1000	1000
Lonicera caerulea L.	Branch	LcB	500	250	500	500	250	>1000	>1000	1000	1000
	Leaves	LcL	1000	>1000	1000	>1000	1000	>1000	>1000	>1000	1000
Berberis amurensis var. quelpaertensis Nakai	Branch	BaB	1000	>1000	>1000	1000	1000	1000	>1000	>1000	1000
	Leaves	BaL	500	>1000	>1000	1000	1000	>1000	>1000	>1000	1000
Ribes maximowiczianum Kom.	Branch	RmB	1000	1000	>1000	1000	500	1000	>1000	>1000	1000
	Leaves	RmL	500	1000	500	1000	500	1000	>1000	1000	1000

MIC values against bacteria and yeast were determined by the serial two-fold dilution method. The growth of the bacteria and yeast were evaluated based on the degree of turbidity of the culture using the naked eye. B.a.: *Bacillus atrophaeus* KACC 14742, K.r.: *Kocuria rhizophila* KACC 14744, M.l.: *Micrococcus luteus* KACC 14819, St.e.: *Staphylococcus epidermidis* KACC 14822, B.s.: *Bacillus subtilis* subsp. Spizizenii KACC 14741, K.p. *Klebsiella pneumoniae* KACC 14816, E.c.: *Enterobacter cloacae* KACC11958, S.e.: *Salmonella enterica* subsp. enterica KACC 10769, P.a.: *Pseudomonas aeruginosa* KACC 10186. MIC: minimum inhibitory concentration

TABLE 5: ALPHA-GLUCOSIDASE INHIBITION ACTIVITIES OF METHANOL EXTRACTS FROM KOREAN NATIVE PLANTS ON MT. HALLA

Plant	Parts	Remark	IC_{50} (μg/mL)*
Prunus padus L.	Branch	PpB	1.0 ± 0.1^{g}
	Leaves	PpL	82.7 ± 4.2^{d}
Lonicera caerulea L.	Branch	LcB	7.2 ± 0.7^{d}
	Leaves	LcL	$>200^{a}$
Berberis amurensis var. quelpaertensis Nakai	Branch	BaB	147.1 ± 10.1^{b}
	Leaves	BaL	90.5 ± 0.6^{c}
Ribes maximowiczianum Kom.	Branch	RmB	27.7 ± 2.1^{f}
	Leaves	RmL	38.4 ± 0.5^{e}

*Amount required for a 50% reduction of α-glucosidase. Each value represents the mean±SD, and the means were significantly different as calculated from a paired Duncan's test at $P<0.05$, a-f means followed with the same letters in each column are not significantly different according to Duncan's multiple range test ($P<0.05$). SD: standard deviation

indicating that *P. padus* should be considered as a useful source for herbal medicine. The variation in pharmaceutical activities between organic extracts indicates that the comparative analysis of the metabolome between branch extracts and leaf extracts will be required for the isolation and characterization of the active compounds in *P. padus*.

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Conflicts of interest:
There are no conflicts of interest.

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