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Aldose Reductase Inhibitory Activity of Methanol Extracts from the Korean Plants

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Abstract – We examined methanol extracts prepared from the Korean plants for their inhibitory effects on rat lens aldose reductase (RLAR) activity *in vitro*. Among 41 plant extracts, the extracts of *Lagerstroemia indica, Punica granatum, Eurya japonica, Liquidambar styraciflua,* and *Vitis coignetiae* exhibited highest inhibitory potency, even more potent than tetramethylene glutaric acid (TMG), known as one of typical aldose reductase inhibitors (IC_{50} value, 0.51 μg/ml). Especially, the extract of *Lagerstroemia indica* (Lythraceae) exhibited the most inhibitory potency (IC_{50} value, 0.069 μg/ml) on RLAR.
Keywords - Korean plant extracts, rat lens aldose reductase, *Lagerstroemia indica*

Introduction

Aldose reductase (AR), the key enzyme in the polyol pathway, has been demonstrated to play important roles not only in the cataract formation in the lens (Van Heyningen, 1959), but also in the pathogenesis of diabetic complications such as neuropathy (Ward, 1973), nephropathy (Beyer-Mears *et al.*, 1984) and retinopathy (Engerman and Kern, 1984). Evidence suggests that compounds that inhibit AR could be effective for the prevention of diabetic complications. A number of structurally diverse naturally occurring and synthetic AR inhibitors have been studied *in vivo* to clarify their effectiveness for prevention of diabetic complications in experimental animals (Beyer-Mears and Cruz, 1985) as well as in clinical trials (Handelsman and Turtle, 1981).

In a series of investigations to evaluate potential AR inhibitors from the Korean plants, we have shown that some hot water extracts from herbal medicines exhibited a significant inhibition of bovine lens (BL) AR *in vitro* (Shin *et al.*, 1993), and a number of flavonoid compounds were isolated and characterized as AR inhibitors from plants (Shin *et al.*, 1994; 1995).

As a preliminary step for evaluations of naturally occurring inhibitors of AR, we tested the effects of methanol extracts of 41 plants on RLAR activity.

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Experimental

Plant materials – Forty one plants from the genus Amaranthaceae, Aquifoliaceae, Campanulaceae, Caprifoliaceae, Compositae, Cornaceae, Cruciferae, Cupressaceae, Cyperaceae, Fagaceae, Ginkgoaceae, Hamamelidaceae, Juglandaceae, Labiatae, Leguminosae, Liliaceae, Lythraceae, Mavaceae, Oleaceae, Pedaliaceae, Pinaceae, Punicaceae, Rosaceae, Theaceae, Ulmaceae, Violaceae and Vitaceae were collected from various regions of Korea. The specimens were authenticated by Emeritus Prof. H.-J. Chi, Seoul National University, Korea.

Preparation of RLAR – Crude RLAR was prepared as follows: rat lenses were removed from Sprague-Dawley rats weighing 250-280 g and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to Hayman and Kinoshita (1965), and then partially purified according to Inagaki *et al.* (1982). Partially purified enzyme with a specific activity of 6.5 mU/mg was routinely used to test enzyme inhibition. The partially purified material was separated into 1.0 ml aliquots and stored at –40°C.

Measurement of RLAR activity – RLAR activities were assayed spectrophotometrically by measuring the decrease in absorption of NADPH at 340 nm over a 4 min period with DL-glyceraldehyde as a substrate (Sato and Kador, 1990). Each 1.0 ml cuvette contained equal units of enzyme, 0.10 M sodium phosphate buffer (pH 6.2), 0.3 mM NADPH with or without 10 mM substrate and inhibitor. The concentration of inhibitors giving 50% inhibition of enzyme activity (IC_{50}) was calculated from the least-squares

regression line of the logarithmic concentrations plotted against the residual activity.

Results and Discussion

Methanol extracts of 41 plants were tested for their inhibitory effect on RLAR and the results were summarized in Table 1. Eleven extracts from plants such as *Ilex integra, Castanopsis cuspidate, Liquidambar styraciflua, Cercis chinensis, Lagerstroemia indica, Picea abies, Punica granatum, Sanguisorba officinalis, Eurya japonica, Liquidambar styraciflua,* and *Vitis coignetiae* were demonstrated to show high degree of inhibition (>80% at 10 μg/ml). Sixteen plant extracts were shown to be below 60% degree of inhibition at the 10 μg/ml that are considered to be far less worthy of further consideration.

To evaluate the AR inhibitory activities between active plants extracts more precisely, their inhibitory potencies and IC_{50} values were estimated and indicated in Table 2. Among them, the extracts of *Lagerstroemia indica, Punica granatum, Eurya japonica, Liquidambar styraciflua,* and *Vitis coignetiae* exhibited highest inhibitory potency, even more potent than tetramethylene glutaric acid (TMG), known as one of typical AR inhibitors (IC_{50} value, 0.51 μg/ml).

Table 1. Effects of plant extracts on RLAR

Family name	Plant name	Part used	AR inhibition* (%)
Amaranthaceae	*Amaranthus mangostanus*	Whole	39.2
Aquifoliaceae	*Ilex integra*	Leaf	81.6
Campanulaceae	*Adenophova triphylla*	Whole	35.3
Caprifoliaceae	*Viburnum awabuki*	Leaf	45.1
Compositae	*Aster scaber*	Whole	67.5
	Siegesbeckia glabrescens	Leaf, Stem	42.3
Cornaceae	*Aucuba japonica*	Leaf, Stem	46.9
Cruciferae	*Cardamine flexuosa*	Whole	33.7
Cupressaceae	*Juniperus chinensis*	Fruit	66.3
	Juniperus rigida	Leaf	73.0
	Thuja orientalis	Leaf, Fruit	77.6
Cyperaceae	*Cyperus amuricus*	Whole	67.5
Fagaceae	*Castanopsis cuspidata*	Leaf	82.5
	Quercus aliena	Leaf	66.0
Ginkgoaceae	*Ginkgo biloba*	Leaf	70.6
Hamamelidaceae	*Liquidambar styraciflua*	Leaf, Stem	94.5
Juglandaceae	*Juglans sinensis*	Leaf	49.8
Labiatae	*Leonurus sibiricus*	Whole	42.8
	Prunella vulgaris	Whole	77.9
	Rabdosia japonica	Whole	66.3
Leguminosae	*Cercis chinensis*	Stem	89.0
	Echinosophora koreensis	Whole	45.2
Liliaceae	*Allium tuberosum*	Root	29.3
	Convallaria keiskei	Whole	27.0
Lythraceae	*Lagerstroemia indica*	Leaf, Stem	98.8
Mavaceae	*Hibiscus mutabilis*	Leaf	47.2
Oleaceae	*Osmanthus heterophylla*	Stem	76.4
Pedaliaceae	*Sesamum indicum*	Whole	44.5
Pinaceae	*Picea abies*	Leaf	87.4
Punicaceae	*Punica granatum*	Fruit	97.2
Rosaceae	*Eriobotrya japonica*	Leaf	54.9
	Prunus persica	Leaf	79.5
Rosaceae	*Raphiolepis umbellata*	Leaf, Stem	75.5
	Sanguisorba officinalis	Whole	94.2
	Sorbus commixta	Leaf, Stem	68.7
Theaceae	*Eurya japonica*	Leaf	96.5
Ulmaceae	*Celtis choseniana*	Leaf	51.8
	Ulmus pumila	Leaf	62.0
	Zelkova serrata	Leaf	68.1
Violaceae	*Viola mandshurica*	Whole	46.6
Vitaceae	*Vitis coignetiae*	Leaf	96.0

– Each sample concentration was 10 μg/ml.
*Inhibition rate was calculated as percentage with respect to the control value.

Table 2. IC_{50} potencies of plant extracts on RLAR

Samples	Concentration (μg/ml)	Inhibition (%)	IC_{50} (μg/ml)
	10	94.7	
TMG*	1	81.7	0.51
	0.1	10.8	
	10	81.6	
Ilex integra	1	50.4	1.41
	0.5	27.6	
	10	67.0	
Aster scaber	5	23.9	7.78
	1	11.4	
	10	66.3	
Juniperus chinensis	5	37.9	5.63
	1	25.4	
	10	73.0	
Juniperus rigida	5	40.4	4.09
	1	31.7	
	10	77.6	
Thuja orientalis	5	61.2	2.97
	1	25.7	
	10	67.5	
Cyperus amuricus	1	43.2	2.61
	0.5	21.4	
	10	82.5	
Castanopsis cuspidata	1	51.6	1.49
	0.5	23.4	
	10	66.0	
Quercus aliena	5	40.3	5.73
	1	17.2	
	10	70.6	
Ginkgo biloba	1	41.4	2.41
	0.5	22.2	
	10	89.0	
Cercis chinensis	1	54.3	1.26
	0.5	24.8	
	1	92.3	
Lagerstroemia indica	0.1	60.1	0.069
	0.05	41.4	
	10	87.4	
Picea abies	1	60.7	1.17
	0.5	23.6	
	10	97.2	
Punica granatum	1	74.5	0.27
	0.1	33.1	
	10	76.4	
Osmanthus heterophylla	5	42.1	4.34
	1	20.5	
	10	79.5	
Prunus persica	1	54.3	1.05
	0.5	36.2	
	10	75.5	
Raphiolepis umbellata	1	30.7	2.60
	0.5	20.4	
	10	94.2	
Sanguisorba officinalis	1	57.0	0.71
	0.5	43.3	
	10	96.5	
Eurya japonica	1	55.5	0.37
	0.1	36.7	
	10	94.5	
Liquidambar styraciflua	1	64.5	0.45
	0.1	26.7	
	10	66.3	
Rabdosia japonica	1	31.3	3.58
	0.5	16.2	
	10	77.9	
Prunella vulgaris	5	46.1	3.39
	1	30.7	
	10	68.7	
Sorbus commixta	5	22.4	7.65
	1	13.2	
	10	62.0	
Ulmus pumila	5	33.7	7.35
	1	21.4	
	10	68.1	
Zelkova serrata	5	40.6	4.72
	1	30.4	
	10	96.0	
Vitis coignetiae	1	65.0	0.21
	0.1	42.6	

Inhibition rate was calculated as percentage with respect to the control value.
*TMG: tetramethylene glutaric acid.

Especially, the extract of *Lagerstroemia indica* (Lythraceae) exhibited the highest inhibitory potency (IC_{50} value, 0.069 μg/ml).

Further experiments are now in progress to characterize active principles from *Lagerstroemia indica*.

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