Inhibitory Effect of Traditional Turkish Folk Medicines on Aldose Reductase (AR) and Hematological Activity, and on AR Inhibitory Activity of Quercetin-3-*O*-methyl Ether Isolated from *Cistus laurifolius* L.

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The inhibitory effects of 40 extracts prepared from 38 traditional Turkish folk medicines on human aldose reductase (h-AR) and hematological activity were investigated. Seven plants containing 5 species of *Cistus* genus exhibited a potent inhibition of h-AR. *Ferulago amani* (root) inhibited the platelet aggregation induced by sodium arachidonate, while *C. laurifolius* (fruit) was found to possess strong inhibition in the blood coagulation assay. An AcOEt extract derived from the leaf of *C. laurifolius* was purified to isolate three known flavonoids. The activity of one, quercetin-3-*O*-methyl ether, was found to be as potent as that of eparlestat, which is known to be a remedy for treating complications associated with diabetes.

Key words *Cistus laurifolius*; complications of diabetes; quercetin-3-*O*-methyl ether; human aldose reductase; platelet aggregation; blood coagulation

Diabetes is a common condition with multiple complications. Aldose reductase (AR), the key enzyme of the polyol pathway, has been demonstrated to play an important role in the etiology of the complications of diabetes. The hyperaggregability[1,2] of platelets, as well as their hypercoagulability[3] in diabetic patients have also been reported to be a cause of chronic diabetic complications. Recently, it has been shown that aspirin,[4] an anti-platelet aggregating drug, or sodium dextran sulfate,[5] an anti-coagulating drug, delay the development of some diabetic complications. Therefore, it is possible that normalization of the aggregability of platelets and coagulability in diabetic patients may be beneficial for the prevention or treatment of chronic diabetic complications. In previous papers, we have reported the results of screening tests involving many plants, Chinese crude drugs, and marine algae, for their inhibitory effects on bovine lens or human AR, platelet aggregation, and blood coagulation in order to examine their possibilities as new natural medicinal resources against the complications of diabetes.[6—9] We also reported the glycation inhibitory activity of style of *Zea mays*[10,11] and *Eisenia bicyclis*[12] for their potential effective treatment of diabetic complications.

In this paper, a total 38 of kinds of traditional Turkish folk medicines, collected from markets in Turkey and in the wild, were examined for three inhibitory activities, human AR activity, platelet aggregation and blood coagulation, to determine a useful drug to treat complications of diabetes. We also studied three known compounds isolated from the leaves of *Cistus laurifolius*, which have already been used for diabetes, as they possess inhibitory effects on h-AR.

MATERIALS AND METHODS

Plant Materials Plant materials, except *Cistus* genus plants, were collected between 1987—1989 in Idris Dagi. Idris Dagi is located in the north-eastern part of Ankara and extends between Kalecik and Akyurt. *Cistus* genus plants were also collected in Ankara, Balikesir, Antalya, Mugla and Izmir. Each material was deposited in the Herbarium of Ankara University. These plant materials were identified by Prof. Dr. Maksut Coşkun as herbarium specimens. Voucher numbers are shown in Table 1.

Extraction of Plant Materials The materials were dried in the shade to be extracted with methanol (MeOH) using the soxhlet extraction method. The extracts were dried in a vacuum drying oven.

Isolation of Compounds from *Cistus laurifolius* Leaves of *C. laurifolius* (155 g), collected from the Sinop area and Boyabat in Turkey in June 1995 (Voucher numbers: T 9501A), were extracted using each organic solvent (1 l×2, 3 h, reflux) to yield the corresponding AcOEt (23 g), MeOH (16 g) and H_2O (15 g) extracts, respectively. The course of purification was monitored by assaying the inhibitory effect on h-AR. The AcOEt extract was dissolved with MeOH and partitioned between *n*-hexane (1 l×5) and MeOH, following which the MeOH soluble part that evaporated off was dissolved with acetonitrile (MeCN) and re-partitioned between *n*-hexane (1 l×5) and MeCN. The MeCN layer was evaporated off *in vacuo*, and the portion (9.46 g) eluted with organic solvent (MeOH, 6 l→acetone, 2 l→AcOEt, 2 l→*n*-hexane, 2 l) by HP20 column chromatography (7.4×20 cm) was partitioned with each 200 ml. Each fraction was simply combined by monitoring the profiles on TLC (silica gel, *n*-hexane/AcOEt, 2 : 1) to yield the seven fractions (I—VII). The first fraction (5.57 g, 200—1800 ml) was re-chromatographed on Sephadex LH-20 (3×100 cm) with MeOH (4 l), and each fraction (60 ml) divided was combined by TLC (silica gel, *n*-hexane/AcOEt, 1 : 1) to give ten fractions (A—J). Fractions I-G (270.8 mg, 1680—1980 ml), which possessed potent activity on h-AR, were purified by HPLC under the following conditions to give quercetin-3-*O*-methyl ether (**1**, 161.6 mg, t_R: 4′30) and quercetin (**2**, 3.3 mg, t_R: 11′12): Column, COSMOSIL 5C18-AR Waters 1×25 cm: Solvent System, H_2O/MeOH (1 : 4): Detector, UV 254 nm:

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Table 1. Screening Data for Inhibitory Effects on Aldose Reductase and Hematological Activity

Plant name	Voucher numbers	Parts	Extraction rate (%)	AR inhibitory activity (%)	Anti-platelet aggregation activity (%)		Anti-blood coagulation activity (%)
					AA	ADP	
Achillea teretifolia Willd.	T8932	Herba	27.1	46	7	18	5
Alyssum sibiricum Willd.	T8705	Herba	20.3	15	3	17	7
Alyssum strigosum Banks & Sol. subsp. *strigosum*	T8813	Herba	13.3	14	11	27	1
Anthemis tinctoria L. var. *tinctoria*	T8931	Herba	16.1	66	12	17	9
Asparagus officinalis L.	T8712	Herba	8.3	20	12	19	0
Astragalus angustifolius Lam. subsp. *angustifolius* var. *angustifolius* subsp. *Pungens* (Willd.) Hayek	T8928	Herba	9.6	29	16	27	−9
Centaurea depressa Bieb	T8930	Herba	11.0	52	24	10	1
Cistus creticus subsp. *creticus* L.	T9902A	Leaf	37.0	90	9	0	5
	T9902B	Fruit	8.0	99	31	42	28
C. laurifolius L.	T9901A	Leaf	46.5	77	1	12	7
	T9901B	Fruit	17.2	108	4	6	>186[a)]
C. monspeliensis L.	T0003A	Leaf	32.1	82	28	12	23
C. parviflorus Lam.	T0005A	Leaf	20.7	94	11	−2	−3
C. salvifolius L.	T0004A	Leaf	32.3	102	16	17	2
Cystopteris fragilis	T8921	Herba	17.4	37	13	9	−5
Ecballium elaterium (L.) A. Rich.	T8708	Herba	23.1	27	15	20	17
Echinophora tournefortii Jaub. & Spach	T8704	Herba	24.0	69	6	7	5
Falcaria vulgaris Bernh.	T8703	Herba	17.7	24	16	3	15
Galium incanum Sm. subsp. *incanum*	T8709	Herba	18.7	19	13	15	4
Heliotropium europeum L.	T8710	Herba	32.1	27	28	34	5
Heracleum paplagonicum Czcczott	T8919	Herba	12.0	17	−3	0	14
Hyoscyamus reticulatus L.	T8933	Herba	7.3	14	6	27	7
Isatis floribunda Boiss. ex Bornm	T8706	Herba	16.6	39	19	29	2
Marrubium astracanicum Jacq. subsp. *astracanicum*	T8916	Herba	17.5	25	11	27	0
Melilotus officinalis (L.) Desr.	T8926	Herba	28.7	19	11	18	6
Mentha longfolia (L.) Hudson subsp. *typhoides* (Briq.) Harley	T8701	Herba	40.2	19	7	0	5
Nepeta muda L. subsp. *nuda*	T8915	Herba	43.7	49	7	11	11
Phlomis pungens Willd. var. *laxiflora* Velen.	T8702	Herba	13.4	33	12	5	8
Plantago coronopus L. subsp. *coronopus*	T8929	Herba	22.2	53	6	0	6
Potentilla recta I.	T8924	Herba	25.0	102	9	12	5
Prunus spinosa L. subsp. *dasyphylla* (Schur) Domin	T8925	Herba	16.4	24	4	−3	35
Scropbularia copolii (Hoppe ex) pers. var. *scopolii*	T8923	Herba	21.6	31	23	22	10
Senecio vernalis Waldst. & Kit	T8711	Herba	5.0	36	9	22	6
Silene alba (Miller) Krause subsp. *divaricata* (Reichb.) Walters	T8922	Herba	15.9	43	55	61	35
Sinapis arvensis L.	T8707	Herba	5.4	23	13	28	−4
Thlaspi arvense L.	T8920	Herba	32.4	31	8	16	2
Thymus sipyleus Boiss. subsp. *sipyleus*	T8917	Herba	15.9	61	6	1	10
Trifolium pannonicum Jacq. subsp. *elongatum* (Willd.) Zoh.	T8927	Herba	19.7	45	17	24	−11
Tripleurospermum callosum E. Hossain	T8914	Herba	23.0	81	18	14	0
Zosima absinthifolia (Vent.) Link	T8918	Herba	25.3	16	20	26	8
Quercetin				72			
Aspirin					93		
Adenosine						98	
Dextran sulfate sodium salt							128

Stimulant activity is expressed by a minus sign (−). *a*) The sample did not coagulate within 999.9 s. Final concentration (F.C.); h-AR (human aldose reductase)=3×10^{-2} unit/ml, AA (arachidonic acid)=0.3 mM, ADP (adenosine diphosphate)=2.0 μM, Samples F.C.; ARI=10 μg/ml, Anti-platelet aggregation effects=500 μg/ml, Anti-blood coagulation=1.8 mg/ml, quercetin: 10 μg/ml, aspirine: 100 μg/ml. adnosine 50 μg/ml, Dextran sulfate sodium salt 50 ng/ml.

Flow Rate, 3.0 ml/min: room temperature. Next, fraction II-F (135.4 mg), one of the fractions exhibiting inhibitory activity, was re-crystallized with MeOH to yield genkwanin (**3**, 3.3 mg) as yellow needles crystals. These compounds were isolated from active fractions.

Effects of Turkish Plant Materials on AR, Platelet Aggregation and Blood Coagulation The inhibitory effects on AR, platelet aggregation and blood coagulation were determined by the methods described in previous papers.[9,11)]

The human muscle AR recombinant activities were assayed spectrophotometrically by measuring the decrease in the absorption of NADPH at 340 nm for 3 min with DL-glyceraldehyde as a substrate. Platelet rich-plasma (PRP) and platelet poor-plasma (PPP) were prepared from the fresh blood of healthy male Japanese white rabbits to measure anti-platelet aggregation and anti-blood coagulation activity. Platelet aggregation was determined by the turbidimetrical method using a platelet aggregometer (Mebanix Corporation: MEBA1 Aggregometer PAM-8T). Coagulation time was evaluated by an automatic recording apparatus (KC-4A, Amelung GmbH, Lemgo) based on the viscosity change of the plasma (PPP). Each sample was tested at least 3 times using the plasma of 3—5 test animals. Epalrestat, aspirin and adenosine sodium dextran sulfate, respectively, were used as positive controls in these assays.

RESULTS AND DISCUSSIONS

The results showed the inhibitory actions of 40 kinds of MeOH extracts obtained by a soxhlet apparatus from Turkish plants on h-AR and hematological activity, including anti-platelet aggregating and anti-coagulating activity (see Table 1). Of the 12 kinds of extracts with inhibitory activity exceeding 60% on h-AR, the MeOH extracts of *Potentilla recta*, *Tripleurospermum callosum*, and *Cistus genus*, *C. laurifolius* (fruit), *C. creticus* (leaf and fruit), *C. monspeliensis* (leaf), *C. salvifolius* (leaf) and *C. parviflorus* (leaf) exhibited potent inhibition in duplicate tests. The MeOH extract of *Silene alba* only inhibited over 60% of platelet aggregation induced by ADP in the platelet aggregating test. However, no sample showed inhibitory activity in the platelet aggregating test induced by sodium arachidonate. Among the 38 species assayed for anti-blood coagulation activity, it was found that only the MeOH extract of *C. laurifolius* (fruit) showed remarkable inhibitory activity (Table 1). We are preparing amounts of the fruit of *C. laurifolius* as experimental material.

The extract derived from the leaf of *C. laurifolius* (20 g) that exhibited an AR inhibitory effect was fractionationed by column chromatography and HPLC, as described in Materials and Methods, to yield three known compounds with monitoring by assay of the inhibitory effect on h-AR. Each compound (Fig. 1), quercetin-3-*O*-methyl ether (**1**) (3-*O*-MQ; 3′,4′,5,7-tetrahydroxy-3-methoxyflavone), quercetin (**2**) and genkwanin (**3**), was identified by comparison of their physical and spectral data with values of authentic samples.

The inhibitory activities of the three compounds isolated are shown in Table 2. The inhibitory activity of 3-*O*-MQ (**1**) was almost as potent as that of the positive control eparlestat, which is a well known remedy for treating complications of diabetes in Japan.[13)]

3-*O*-MQ (**1**) has been reported to be highly active against a wide range of human picornaviruses,[14,15)] platelet aggregation,[16)] and histamine-induced tracheal relaxation[17)] *in vitro*. The structure–activity relationships of many flavonoid derivatives such as flavone, flavonol and chalcone on recombinant human aldose reductase, bovine lens aldose reductase, and rat lens aldose reductase have been described.[18—20)] However, these studies have not referred to 3-*O*-MQ (**1**) at all. L. Van Hoof *et al.* reported that no signs of toxicity were observed in mice *in vivo*.[21)]

As presented above, **1** is thought to be a promising aldose reductase inhibitor which warrants further investigation.

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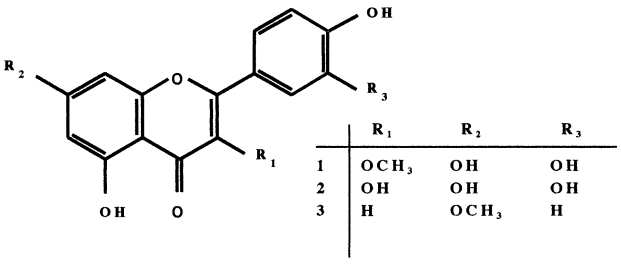


Fig. 1. Structures of the Isolated Compounds **1**—**3**

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Table 2. Inhibitory Effect of Isolated Compounds on h-AR

	Inhibition (%)						IC_{50} (μg/ml)	IC_{50} (M)
	3 (μg/ml)	1	0.3	0.1	0.03	0.003		
Quercetin-3-*O*-methyl ether (**1**)	92	—	74	38	17	—	1.3×10^{-1}	4.1×10^{-7}
Quercetin (**2**)	71	47	17	—	—	—	1.2	4.0×10^{-6}
Genkwanin (**3**)	25	—	—	—	—	—	—	—
Epalrestat	—	—	95	—	52	0	3.2×10^{-2}	1.0×10^{-7}

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