

# Antihypercholesterolaemic and antioxidant activity assessment of some plants used as remedy in Turkish folk medicine

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## Abstract

Ethanollic and aqueous extracts from five plant species used in Turkish traditional medicine were evaluated for in vivo hypercholesterolaemic and antioxidant activities: *Agrostemma githago* L., *Potentilla reptans* L., *Thymbra spicata* var. *spicata* L., *Urtica dioica* L. and *Viscum album* var. *album* L. We assayed the effects of the administration of plant extracts on serum total cholesterol, triglyceride, HDL-C, LDL-C, glucose, AST and ALT concentrations in mice fed with cholesterol-rich diet. In addition, plasma TAA, MDA and NO<sub>x</sub> levels in the same animals were assayed. All the aqueous plant extracts did not affect the serum cholesterol concentration. However, the ethanolic extracts of *Agrostemma githago*, *Thymbra spicata* and *Viscum album* decreased the serum cholesterol concentration in the mice fed with high-cholesterol diet without inducing any gastric damage. The ethanolic extracts of *Thymbra spicata*, *Viscum album*, *Potentilla reptans* and *Urtica dioica* and the aqueous extract of *Agrostemma githago* increased the serum HDL concentration, whereas the ethanolic extracts of *Agrostemma githago*, *Thymbra spicata*, *Viscum album* and *Urtica dioica* decreased the serum LDL-C concentration. *Thymbra spicata* and *Viscum album* were observed to decrease the serum triglyceride concentration. Among the plant extracts studied, the ethanolic extracts of *Thymbra spicata* significantly decreased the MDA level in mice. The ethanolic extract of *Potentilla reptans* increased in NO<sub>x</sub>. None of these plants showed statistically prominent activity on plasma TAA. Results of the present study indicated that the ethanolic extracts of *Agrostemma githago*, *Thymbra spicata* and *Viscum album* showed potent hypocholesterolaemic activity in the mice fed with a diet containing high-cholesterol.

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**Keywords:** Antihypercholesterolaemic effect; *Agrostemma githago*; *Potentilla reptans*; *Thymbra spicata*; *Urtica dioica*; *Viscum album*; Hypercholesterolaemia; Antioxidant activity

## 1. Introduction

Cholesterol, a representative sterol and a component of all eucaryotic plasma membranes, is necessary for the growth and viability of higher organisms. However, an increased level of cholesterol, hypercholesterolaemia, is known a risk factor for development of cardiovascular diseases including atherosclerosis, myocardial infarction and cerebral paralysis (Child and Kuksis, 1983). Hypercholesterolaemia enhances the free radical generation in various ways (Prasad and Kalra, 1993) and the formation of oxygen free radicals (OFR), such as superox-

ide anion radical (O<sub>2</sub><sup>-</sup>) or peroxyntirite (ONOO<sup>-</sup>) is postulated to be derived from different cellular sources in the vasculature and in parenchymatous tissues, play a significant role in the pathogenesis of many other diseases besides cardiovascular diseases, i.e., cancer and inflammatory disorders as well (Das et al., 2000).

Hypercholesterolaemia is among the most common health problems treated with traditional remedies. Therefore, it is crucial to evaluate the potential of herbal remedies for the discovery of novel bioactive compounds that might serve as leads for the development of potent drugs. In our ongoing research project on the medicinal plants used in Turkish traditional medicine for the treatment of hypercholesterolaemia, we undertook the present screening study in order to reveal and elucidate traditional use of these plants from the view of scientific point.

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Plant materials are selected based on the literature data available for the relevant effects in the Turkish traditional medicines. *Potentilla reptans* aerial parts are used to treat blood circulation troubles (Baser et al., 1986), *Thymbra spicata* leaves have recently gained much popularity as a remedy to combat hypercholesterolaemia (Baser et al., 1986), *Urtica dioica* herbs are used as panacea for every kind of ailments (Baser et al., 1986), *Viscum album* herbs are described as prophylactic in atherosclerosis, i.e., infusion of one spoonful drug in 1 l of water is taken one glassful after meals (Baser et al., 1986). Among the plants studied in the present study only *Agrostemma githago* remained outside the selection criteria. Although do not possess any application in Turkish folk medicine for the relevant purpose, due to the rich saponin content, its extracts were studied for the potential effects on cholesterol levels. In order to prove the activity of these plant remedies, ethanol and aqueous extracts were prepared and were experimentally tested in mice for their effects on various serum biochemical parameters.

## 2. Materials and methods

### 2.1. Plant materials

Plant materials were collected from different localities in Turkey. Voucher specimens were authenticated by Prof. Dr. Hayri Duman of Department of Biology, Faculty of Science & Art, Gazi University, and were deposited in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey. Collection sites, parts used and herbarium numbers of plant materials are described below.

*Agrostemma githago* L. (Caryophyllaceae): Afyon, Suhut, hills behind town, aerial parts [GUE 2364].

*Potentilla reptans* L. (Rosaceae): Ankara, Kızılcahamam, Güvem village, aerial parts [GUE 2365].

*Thymbra spicata* L. var. *spicata* P.H.Davis (Lamiaceae): Urfa, aerial parts [GUE 2366].

*Urtica dioica* L. (Urticaceae): Amasya, Suluova, Kurnaz village, aerial parts [GUE 2367].

*Viscum album* L. var. *album* (Loranthaceae): Amasya, Suluova, Fındıklı village, whole plant [GUE 2368].

### 2.2. Preparation of plant extracts

Each plant material was dried under shade and powdered to a fine grade by using a laboratory scale mill. The plant parts and the extract yields (w/w) are given below. The extracts were prepared as described below:

Ethanol (EtOH) extract: Dried plant material (10 g) was extracted with 90% EtOH at room temperature for two times ( $\times 200$  ml). The combined ethanolic layers were evaporated to dryness in vacuo to give crude EtOH extract.

Aqueous (H<sub>2</sub>O) extract: Dried plant material (10 g) was extracted with distilled water at room temperature for two times ( $\times 200$  ml). The combined aqueous layers were lyophilized to give the crude H<sub>2</sub>O extract.

EtOH and aqueous extract yields, respectively, for the plant materials were as follows (w/w, %): *Agrostemma githago*: 19.11/19.88; *Potentilla reptans*: 31.36/26.11; *Thymbra spicata*: 18.29/29.60; *Urtica dioica*: 22.54/48.82; *Viscum album*: 27.19/41.56.

### 2.3. Animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals were left for 2 days for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum. A minimum of 10 animals were used in each group.

All the plant extracts were given orally to test animals in 100 mg/kg doses after suspending in a mixture of distilled H<sub>2</sub>O and 0.5% sodium carboxymethyl cellulose (CMC) by using a gastric gavage. Two control groups were employed in the study. The hypercholesterolaemic group (positive control) animals received the same experimental handling as those of the test groups, i.e., with hypercholesterol diet, except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. The negative control group animals were maintained on standard pellet diet and water ad libitum, without administering any plant extract. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

### 2.4. Induction of hypercholesterolaemia and experimental protocols

Hypercholesterolaemia was induced in animals by feeding powdered fodder containing 1% cholesterol for 30 days. All animals had free access to food and water ad libitum during the experimental period. The test group animals concurrently received plant extracts except for control mice every morning. At the end of 30 days experimental period, blood samples were collected from the mice in all groups for the biochemical determinations.

Serum high-density lipoprotein cholesterol (HDL-C) levels were determined using Crescent Diagnostics Cholesterol test kit after precipitation of apolipoprotein B containing lipoproteins by phosphotungstic acid and magnesium chloride. The cholesterol content of low-density lipoprotein (LDL-C) was extrapolated using the Friedwald equation. Alanine aminotransferase (ALT or SGPT) and aspartate aminotransferase (AST or SGOT) levels were determined by using commercial kits Teco Diagnostics assay kit. Serum total protein, triglycerides and cholesterol values were measured with commercially available assay kits (Teco Diagnostics, CA, USA) by enzymatic methods. Malondialdehyde (MDA) was estimated according to method of Draper and Hardley (1990), which is based on the coupling MDA with thio-barbituric acid. Plasma total antioxidant activity (TAA) was measured according to Koracevic et al. (2001). Briefly, a standardized solution of Fe–EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, leading to the formation of hydroxyl radicals ( $\bullet$ OH). These reac-

tive oxygen species degrade benzoate, resulting in the release of TBARS. Antioxidants from the added sample cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of color development defined as the TAA. Nitric oxide metabolites (nitrates + nitrites,  $\text{NO}_x$ ) were assayed in plasma by colorimetric method of Griess (Miranda et al., 2001).

### 2.5. Gastric-ulcerogenic effect

Since the plant extracts were administered for a long period (30 days), potential risks for gastric injury were also evaluated. For this purpose, after finishing the hypocholesterolemic activity experiment, mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through the greater curvature and examined under dissecting microscope for lesions or bleedings.

### 2.6. Statistical analysis of data

Data obtained from animal experiments were expressed as mean standard error ( $\pm$ S.E.M.). Statistical differences between the high-cholesterol diet and the control diet, and high-cholesterol diet and plant extracts given orally were evaluated by ANOVA and Student's–Newman–Keuls post hoc tests. A difference in the mean values of  $P < 0.05$  was considered to be significantly significant [ $^*P < 0.05$ ;  $^{**}P < 0.01$ ;  $^{***}P < 0.001$ ].

## 3. Results and discussion

In the present study, five plant species which were selected according to the ethnobotanical information gathered from Turkish folk medicine were evaluated for their in vivo antihypercholesterolaemic and antioxidant activities. Two kinds of extracts

with ethanol and water were prepared from each plant material and their inhibitory effects on hypercholesterolaemia and other related serum parameters in mice were examined. The results of in vivo experiments are shown in Tables 1 and 2.

Cholesterol is one of the body's fats. Cholesterol and triglyceride are important building blocks in the structure of biological membranes, and also used in the biosynthesis of steroid hormones, bile acids and Vitamin D as well as in producing energy. However, the high-cholesterol concentration in the blood increases the risk of developing atherosclerosis and related cardiovascular diseases. Cholesterol concentration in the blood has been affected by both the cholesterol content of the diet and the cholesterol synthesized in the liver (Noyan, 1996). In the present study, the mice fed with high-cholesterol diet had higher ( $P < 0.05$ ) the serum total cholesterol concentration than the mice fed with normal diet (66.7% higher). Among the plant extracts studied, the ethanol extracts of *Agrostemma githago*, *Thymbra spicata* and *Viscum album* decreased ( $P < 0.01$ ) the serum cholesterol concentration in the mice fed with high-cholesterol diet 55, 57.1 and 59.1%, respectively, without inducing any gastric damage. Whereas the aqueous extracts of all plants did not affect the serum cholesterol concentration in remarkable degrees. Although not significant ethanol extract of *Urtica dioica* also showed some inhibitory activity (42.2%) (Table 1).

On the other hand, low-density lipoprotein (LDL) takes the cholesterol from liver to tissues, whereas high-density lipoprotein (HDL) facilitates the translocation of cholesterol from the peripheral tissues to liver for catabolism. Therefore, HDL has a useful effect in reducing tissue cholesterol, and increasing ratio in serum is suggested, while decreasing level that for LDL-cholesterol to reduce the risk of cardiovascular diseases (Nofer et al., 2002). As shown in Table 1, all the plant extracts studied showed some increasing activity on serum

Table 1  
Effects of the test materials on serum biochemical parameters: total-, HDL-, and LDL-cholesterol and triglyceride

Material	Extract type	Dose (mg/kg)	Total cholesterol (mg/dl) (%)	HDL-C (mg/dl) (%)	LDL-C (mg/dl) (%)	Triglyceride (mg/dl) (%)
Control group			131.0 $\pm$ 13.2	29.2 $\pm$ 1.3	91.4 $\pm$ 20.6	122.7 $\pm$ 29.8
Hypercholesterolaemic group			218.4 $\pm$ 16.3 <sup>#</sup>	25.8 $\pm$ 0.8	143.0 $\pm$ 25.3	194.2 $\pm$ 27.2
<i>Agrostemma githago</i>	E	100	<b>98.2 <math>\pm</math> 11.2<sup>**</sup></b> (–55.0)	36.8 $\pm$ 2.9 (+29.9)	<b>42.0 <math>\pm</math> 12.0<sup>*</sup></b> (–70.6)	117.8 $\pm$ 5.0 (–39.3)
	W	100	194.0 $\pm$ 30.0 (–11.2)	<b>41.5 <math>\pm</math> 1.8<sup>**</sup></b> (+37.8)	197.4 $\pm$ 22.5 (+38.0)	102.6 $\pm$ 21.5 (–47.2))
<i>Potentilla reptans</i>	E	100	192.8 $\pm$ 12.4 (–11.7)	<b>38.6 <math>\pm</math> 2.7<sup>*</sup></b> (+33.2)	113.2 $\pm$ 17.3 (–20.8)	225.4 $\pm$ 31.6 (+16.1)
	W	100	153.0 $\pm$ 25.9 (–29.9)	33.2 $\pm$ 2.3 (+22.3)	101.8 $\pm$ 23.4 (–28.8)	111.5 $\pm$ 46.0 (–42.6)
<i>Thymbra spicata</i>	E	100	<b>93.6 <math>\pm</math> 10.0<sup>**</sup></b> (–57.1)	<b>43.6 <math>\pm</math> 3.7<sup>**</sup></b> (+40.8)	<b>62.4 <math>\pm</math> 7.7<sup>*</sup></b> (–56.4)	<b>45.6 <math>\pm</math> 10.9<sup>**</sup></b> (–76.5)
	W	100	168.0 $\pm$ 26.9 (–23.1)	32.8 $\pm$ 2.1 (+21.3)	106.6 $\pm$ 25.1 (–25.5)	87.6 $\pm$ 19.7 (–54.9)
<i>Urtica dioica</i>	E	100	126.2 $\pm$ 18.5 (–42.2)	31.0 $\pm$ 2.2 (+16.8)	<b>48.6 <math>\pm</math> 14.2<sup>*</sup></b> (–66.0)	189.8 $\pm$ 19.7 (–2.2)
	W	100	160.0 $\pm$ 20.4 (–26.7)	<b>39.8 <math>\pm</math> 4.0<sup>*</sup></b> (+35.2)	90.2 $\pm$ 24.6 (–36.9)	150.0 $\pm$ 41.4 (–22.8)
<i>Viscum album</i>	E	100	<b>89.3 <math>\pm</math> 5.9<sup>**</sup></b> (–59.1)	<b>48.4 <math>\pm</math> 4.8<sup>**</sup></b> (+46.7)	<b>24.3 <math>\pm</math> 6.6<sup>**</sup></b> (–83.0)	<b>76.3 <math>\pm</math> 7.3<sup>*</sup></b> (–60.7)
	W	100	139.4 $\pm$ 44.3 (–36.2)	34.4 $\pm$ 1.6 (+25.0)	63.8 $\pm$ 23.4 (–55.4)	<b>63.6 <math>\pm</math> 9.5<sup>*</sup></b> (–67.3)

(S.E.M.), mean standard error; (+) increase/stimulatory rate; (–) decrease/inhibitory rate from hypercholesterolaemic animals; (E) ethanol extract; (W) aqueous extract.

<sup>\*</sup>  $P < 0.05$  significant from hypercholesterolaemic control.

<sup>\*\*</sup>  $P < 0.01$  significant from hypercholesterolaemic control.

<sup>\*\*\*</sup>  $P < 0.001$  significant from hypercholesterolaemic control.

<sup>#</sup>  $P < 0.05$  significant from control animals.

Table 2  
Effects of the test materials on serum biochemical parameters on glucose, AST, ALT, MDA, TAA and nitric oxide

Material	Extract type	Dose (mg/kg)	Glucose (mg/dl) (%)	AST (IU/L) (%)	ALT (IU/L) (%)	MDA (nmol/mL) (%)	TAA (mmol/L) (%)	Nitric oxide ( $\mu\text{mol/L}$ ) (%)
Control group			95.7 $\pm$ 19.2	58.3 $\pm$ 13.9	12.8 $\pm$ 5.7	1.8 $\pm$ 0.70	1.10 $\pm$ 0.52	26.54 $\pm$ 3.13
Hypercholesterolaemic group			79.8 $\pm$ 10.8	75.8 $\pm$ 14.6	41.4 $\pm$ 10.5	3.5 $\pm$ 1.80	0.80 $\pm$ 0.37	21.80 $\pm$ 3.21
<i>Agrostemma githago</i>	E	100	61.0 $\pm$ 11.7 (-23.6)	76.3 $\pm$ 12.8	10.9 $\pm$ 13.3 (-73.7)	2.55 $\pm$ 0.86 (-27.2)	0.86 $\pm$ 0.33 (+6.8)	24.68 $\pm$ 4.17 (+11.7)
	W	100	111.6 $\pm$ 7.8 (+39.8)	53.8 $\pm$ 12.9 (-29.0)	34.8 $\pm$ 15.6 (-15.9)	2.11 $\pm$ 0.90 (-39.6)	0.99 $\pm$ 0.16 (+23.3)	41.26 $\pm$ 9.35 (+47.2)
<i>Potentilla reptans</i>	E	100	113.6 $\pm$ 21.2 (+42.3)	<b>13.0 <math>\pm</math> 3.3*</b> (-82.8)	<b>8.0 <math>\pm</math> 3.1*</b> (-80.7)	2.34 $\pm$ 0.79 (-33.2)	0.97 $\pm$ 0.37 (+20.5)	<b>49.36 <math>\pm</math> 3.42*</b> (+55.8)
	W	100	105.6 $\pm$ 26.3 (+32.3)	45.2 $\pm$ 14.1 (-40.4)	25.4 $\pm$ 10.3 (-38.6)	3.51 $\pm$ 1.46	0.69 $\pm$ 0.53 (-13.7)	30.52 $\pm$ 10.38 (+28.6)
<i>Thymbra spicata</i>	E	100	47.8 $\pm$ 8.4 (-40.1)	29.6 $\pm$ 9.3 (-60.9)	16.8 $\pm$ 7.4 (-59.4)	<b>1.24 <math>\pm</math> 0.35*</b> (-64.5)	0.99 $\pm$ 0.16 (+23.3)	24.75 $\pm$ 3.89 (+11.9)
	W	100	119.8 $\pm$ 25.6 (+50.1)	71.7 $\pm$ 10.9 (-5.8)	12.3 $\pm$ 2.5 (-70.3)	2.54 $\pm$ 0.69 (-27.5)	0.97 $\pm$ 0.25 (+20.5)	37.13 $\pm$ 4.63 (+41.3)
<i>Urtica dioica</i>	E	100	69.8 $\pm$ 8.3 (-12.5)	<b>21.5 <math>\pm</math> 6.8*</b> (-71.6)	<b>8.2 <math>\pm</math> 3.2*</b> (-80.2)	2.55 $\pm$ 0.69 (-27.2)	0.97 $\pm$ 0.33 (+20.5)	27.64 $\pm$ 3.27 (+21.1)
	W	100	84.0 $\pm$ 14.4 (-5.5)	44.4 $\pm$ 10.4 (-41.4)	20.8 $\pm$ 7.1 (-49.8)	1.99 $\pm$ 0.79 (-43.0)	0.82 $\pm$ 0.41 (+2.7)	23.82 $\pm$ 6.18 (+8.5)
<i>Viscum album</i>	E	100	59.4 $\pm$ 6.8 (-25.6)	32.8 $\pm$ 10.5 (-56.7)	21.0 $\pm$ 7.8 (-49.3)	2.72 $\pm$ 1.05 (-22.3)	1.04 $\pm$ 0.12 (+30.1)	22.81 $\pm$ 2.22 (+4.4)
	W	100	54.6 $\pm$ 12.2 (-31.6)	49.6 $\pm$ 13.6 (-34.6)	34.0 $\pm$ 14.8 (-17.9)	<b>1.74 <math>\pm</math> 0.56*</b> (-50.2)	0.69 $\pm$ 0.62 (-13.7)	21.02 $\pm$ 5.06

(S.E.M.), mean standard error; (+) increase/stimulatory rate; (-) decrease/inhibitory rate from hypercholesterolaemic animals; (E) ethanol extract; (W) aqueous extract.

\*  $P < 0.05$  significant from hypercholesterolaemic control.

\*\*  $P < 0.01$  significant from hypercholesterolaemic control.

HDL-C level, but the effects of aqueous extracts of *Urtica dioica* ( $P < 0.05$ ) and *Agrostemma githago* ( $P < 0.01$ ) and the ethanolic extracts of *Viscum album* ( $P < 0.001$ ), *Thymbra spicata* ( $P < 0.01$ ) and *Potentilla reptans* ( $P < 0.05$ ) were significant. Meanwhile, serum LDL-C levels of mice were decreased by the ethanolic extracts of *Urtica dioica* ( $P < 0.05$ ), *Thymbra spicata* ( $P < 0.05$ ), *Agrostemma githago* ( $P < 0.05$ ), and *Viscum album* ( $P < 0.01$ ) (Table 1). Serum triglyceride levels were also significantly inhibited by the ethanol extract of *Thymbra spicata* ( $P < 0.01$ ) and both extracts of *Viscum album* ( $P < 0.05$ ).

If it is taking into account the serum concentrations of total cholesterol, HDL, LDL and triglyceride in the hypercholesterolaemic mice, our results indicated that the ethanolic extracts of *Thymbra spicata* and *Viscum album* possess a favorable effect in the management of these parameters and eventually reducing of the risk of atherosclerosis and relevant cardiovascular diseases.

*Agrostemma githago* is reported to contain saponins (Siepmann et al., 1998). A number of studies have shown that saponins lowered the serum cholesterol levels in a variety of animals including human subjects (Southon et al., 1988). Since saponins form micelles with sterols such as cholesterol and bile acids, they may provide a depletion of body cholesterol by preventing its reabsorption and increasing its excretion (Sidhu and Oakenfull, 1986). Therefore, the cholesterol-lowering effect of the ethanolic extracts of *Agrostemma githago* may be attributed to saponins in the present study.

*Thymbra spicata* is known as rich source of terpenoids, in addition to a variety of iridoid glycosides and flavonoids are accumulated in considerable amounts. Volatile oil of the plant was reported to contain very rich with respect to its carvacrol content (Hanci et al., 2003). Isoprenoids such as thymol, carvacrol and ionone have been shown to lower plasma cholesterol concentrations in chickens. The structural diversity of the isoprenoids which suppress cholesterol synthesis may be reconciled by their ability to increase pyrophosphatase activity, thus leading to the production of the endogenous, post-transcriptional regulator of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (Case et al., 1995).

*Viscum album* has been used in the indigenous system of medicine for the treatment of various diseases including atherosclerosis and hypertension (Wagner et al., 1986). Generally represented compounds include the flavonoids (quercetin), terpenoids ( $\beta$ -amyrin, resin acids, beta-sitosterol, stigmasterol, sterol A), amines and phenolic compounds (Newall et al., 1996).

Although cholesterol is the major sterol presented in animals and plants generally contain a mixture of sterols, with campesterol and sitosterol predominating. Different mechanisms have been suggested to explain the cholesterol-lowering activity of plant sterols and stanols (Meguro et al., 2001). Plant sterols, campesterol and sitosterol, are structurally similar to cholesterol. They may displace cholesterol from mixed micelles (Child and Kuksis, 1986) because they are more hydrophobic than cholesterol. This replacement causes a reduction of micellar cholesterol concentrations and consequently lowers cholesterol absorption (Child and Kuksis, 1983).

Hypercholesterolaemia caused fatty liver and eventually elevation of liver enzymes (Choe et al., 2001). Therefore, in the



present study several serum parameters, other than cholesterol, were also evaluated in order to reveal the antioxidant profile of the extracts as well as effects on hepatic markers and glucose. These parameters would also be helpful for the assessment of mechanism of effect and possible risks or toxicity profile. None of these plants were found to be statistically significant in serum glucose levels, but the inhibitory rates were notable for ethanol extract of *Thymbra spicata* (40.1%), for aqueous (31.6%) and for ethanol extracts of *Viscum album* (25.6%). Recently a significant hypoglycaemic effect as well as antioxidant activity (evaluated by tissue MDA and glutathion levels) of three subspecies of *Viscum album* in streptozotocin-diabetic rats was reported (Orhan et al., 2005). Although, notable inhibitory effects were observed for almost all plant extracts on hepatic marker enzymes, i.e., serum AST (except those of *Agrostemma githago* ethanol and *Thymbra spicata* water extracts) and ALT levels, only those of ethanol extracts from *Urtica dioica* and *Potentilla reptans* were statistically remarkable, which might be attributed to antihepatotoxic potential of the extracts.

Turkdogan et al. (2003) observed the therapeutic effectiveness of *Urtica dioica* in the prevention liver fibrosis and cirrhosis. They suggested that hepatoprotective properties of *Urtica dioica* was possibly through immunomodulator and antioxidant activities. Similar to results, Kanter et al. (2003) also suggested that *Urtica dioica* decrease the lipid peroxidation and liver enzymes (AST), and increase the antioxidant defence system activity in the carbon tetrachloride-treated rats.

It is suggested that hypercholesterolaemia increases the level of lipid peroxidation in the serum of hypercholesterolaemic rabbits and guinea pigs (Das et al., 2000). Szczeklik et al. (1985) also observed an increase in plasma MDA levels in rabbits that consumed a high-cholesterol diet. As shown in Table 2, the level of lipid peroxidation in cholesterol-rich group was comparatively higher than the control group of animals. Meanwhile, except that of *Potentilla reptans* water extract was completely inactive, all extracts showed more than 20% inhibitory activity on lipid peroxidation. However, the MDA concentration was significantly decreased ( $P < 0.05$ ) by the ethanolic extract of *Thymbra spicata* and aqueous extract of *Viscum album*.

The total antioxidant activity of plasma was depleted in cholesterol-rich diet group compared to control group but not significantly. None of these plant extracts showed statistically remarkable effect in restoring the reduced plasma TAA level, but generally they had tendency to normalize. But, contrarily, the aqueous extracts of *Potentilla reptans* and *Viscum album* completely reversed the effect.

Hypercholesterolaemic diet induced a small reduction on the plasma concentration of NO metabolites and ethanolic extract of *Potentilla reptans* significantly increased the NO level ( $P < 0.05$ ). This effect might be due to the tannin content of *Potentilla* species. Some tannin derivatives from traditional herbs were known to be effective on HMG-CoA reductase inhibitors which might be effective on hypercholesterolaemia (Chang et al., 2001). Although not significant, aqueous extracts from *Agrostemma githago*, *Thymbra spicata*, *Potentilla reptans* and ethanol extract of *Urtica dioica* showed notable increasing

activity. On the other hand, water extract of *Viscum album* was completely inactive.

Kang et al. (1997) observed the activity of nitric oxide synthase (NOS) in plasma of high-fat diet (HFD, 2% cholesterol and 100 g table butter/kg diet) and they suggested that through antioxidant supplementation, i.e., selenium a significant reduction in serum cholesterol was observed as well as in plasma NOS activity relative to HFD fed animals.

Conclusively, the observed cholesterol-reducing actions of the ethanolic extracts of *Thymbra spicata* and *Viscum album* indicates that these plants extracts possess some potential medicinal value and explain their ethnomedical use. The in vivo hypocholesterolemic activity of all the plant remedies investigated in the present study is reported here for the first time. This aspect together with isolation and chemical characterization of the plant extract responsible for hypocholesterolemic and antioxidant activity merits further study. Our studies on the hypocholesterolemic activity of the aforementioned plant remedies are in progress for the isolation of active constituents and to elucidate their mode of action.

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