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Inhibitory Activity of Some Plant Methanol Extracts on 3-Hydroxy-3-Methylglutaryl Coenzyme a Reductase

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Abstract: Beta-hydroxy-beta-methylglutaryl coenzyme a reductase (HMG CoA reductase) is the key enzyme in cholesterol biosynthesis. Inhibition of this enzyme reduces the synthesis of cholesterol and could be used in the management of coronary artery disease. Drugs used for the management of cholesterol biosynthesis have showed some side effects that are cause of new trends in the nature. This study was designed to find new HMG CoA reductase inhibitors from natural resources. One hundred plants were botanically identified and their methanol extracts were prepared. Anti HMG-CoA reductase activity of the extracts were determined spectrophotometrically by NADPH oxidation, using HMG-CoA as the substrate. *Quercus infectoria, Rosa damascena* and *Myrtus communis* extracts showed more than 50% inhibitory effect on the enzyme activity and 21 extracts showed an inhibitory effect between 30-50 percent on activity of HMG-CoA reductase. Kinetic study of the enzyme was performed in the presence of two concentrations of the effective extracts (0.05 and 0.15 mg mL^{-1}). These active extracts showed non-competitive inhibition by Lineweaver-Burk plot analysis. Under the standard condition, Km value for enzyme was 0.6 mM and V_{max} value was 0.011 mM min^{-1}. When 0.15 mg mL^{-1} of extracts of Quercus infectoria, Rosa damascena and Myrtus communis were used the V_{max} values of 0.0041, 0.0031 and 0.0028 mM min^{-1} were obtained, respectively. Therefore, purification and characterization of their active constituents and *in vivo* examination of these active extracts, is necessary in order to be used as safer therapeutic agents in the future.

Key words: HMG-Co A reductase, *Quercus infectoria*, *Rosa damascena*, *Myrtus communis*, Non-competitive inhibition

INTRODUCTION

Cholesterol is a sterol and a constituent of all eukaryotic plasma membrane. It is essential for the growth and viability of higher organisms. Maintenance of cholesterol homeostasis is very important for healthy statue and is accomplished by a regulatory complex network (Avci *et al*., 2006; Deng, 2009). However, hypercholesterolemia is one of the known risk factors in the development of Coronary Artery Disease (CAD) and enhances free radical generation which plays an important role in the pathogenesis of other diseases besides cardiovascular diseases such as cancer and inflammatory disorders. Epidemiological studies have shown a continuous relationship between total cholesterol and Coronary Heart Disease (CHD) (Avci *et al*., 2006; Parab and Mengi, 2002). CHD is the leading cause of death in developing countries, where it is responsible for more than 50% of all mortalities (Faergeman, 2000). Elevated plasma cholesterol level also promotes other debilitating diseases including certain forms of cancer, diabetes and obesity. Therefore, hypercholesterolemia and its associated cardiovascular diseases represent one of the greatest worldwide economic, social and medical challenges that we are facing now (Hui and Howles, 2005; Deng, 2009).

HMG-CoA reductase reaction is the rate limiting step in the biosynthesis of cholesterol and isopernoides and is a unique molecular target in anti cancer therapy (Wong *et al*., 2002; Bochar *et al*., 1997). Drugs that lower cholesterol level have been used for decades, but the high prevalence of their adverse effects such as myopathy, liver damages and potential drug-drug interactions has been reported too (Deng, 2009). Therefore, finding and

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development of other therapeutic agents for controlling cholesterol level especially safer agents are is warranted. Plants are the best resources of new drug agent and their use for medicinal purposes has a long history (Deng, 2009).

The existence of cholesterol lowering agents and HMG-CoA reductase inhibitors have been demonstrated in different plant species including Garlic (Chetty *et al*., 2003), *Morus alba*, *Melissa officinalis*, *Artemisia capillaries* (Lee *et al*., 2008), *Vitis vinifera* (Koo *et al*., 2008), *Ananas comosus* (Xie *et al*., 2007), Kiwifruit (Jung *et al*., 2005) and *Gynostemma pentaphyllum* (Megalli *et al*., 2005). However, more searches for finding the most effective HMG-CoA reductase inhibitors from natural sources are needed. In the present study, we have screened methanol extracts of various plants for their anti- HMG-CoA reductase activity to find safer and cheaper agents which may be used as medications in future for prevention and control of hypercholesterolemia and related diseases.

MATERIALS AND METHODS

Reagents: HMG-CoA reductase and HMG-CoA were obtained from SIGMA and NADPH purchased from Fluka, USA. All other reagents were analytical grade.

Plants: Different parts of plants were collected from various regions of Kerman province. Plants were botanically identified by Dr. Mirtajaldini, Department of Botany, Bahonar University, Kerman, Iran during 2008-2009 (Table 1). A voucher specimen of each plant was deposited at the herbarium of the Herbal Medicines Research Center, Faculty of Pharmacy, Kerman University of Medical Sciences. Plant materials were air dried in dark place and grounded into fine powder. The powdered material (20 g) was extracted with 200 mL of absolute methanol for 24 h. The suspensions were filtered and air-dried; these air-dried samples were stored at -20°C in dark vials until use (Gholamhoseinian *et al*., 2009; Sharma *et al*., 2005).

Enzyme assay: HMG-CoA reductase activity was measured spectrophotometrically. The HMG-CoA dependent oxidation of NADPH was measured in 340 nm and pH 7.5; 10 μL of each preparation containing 50 μg crude extract was added to the reaction mixture including HMG-CoA (1.2 mmol L^{-1}), NADPH (1.2 mmol L^{-1}), HMG-CoA reductase (1 unit), 0.1 mol L^{-1} potassium phosphate buffer containing (400 mM KCl, 0.1 mg mL^{-1} of bovine serum albumin and 3.5 mmol L^{-1} EDTA) (final volume of 1 mL). Absorbance reduction at 340 nm was measured in 6 min interval (Xie *et al*., 2007). HMG-CoA reductase inhibitory activity was calculated by using the following formula (Jung *et al*., 2005):

$$\text{Inhibition}\% = \frac{\Delta \text{ Absorbance control} - \Delta \text{ Absorbance test}}{\Delta \text{ Absorbance control}} \times 100$$

Kinetic study: In order to determine the kinetic properties of the HMG-CoA reductase after addition of methanol extracts of *Quercus infectoria*, *Rosa damascena* and *Myrtus communis*, the activity was assayed by using various concentrations of HMG-CoA (0.3, 0. 6, 0. 9 and 1.2 mmol L^{-1}) in the absence and presence of two different concentrations of the extracts (0.05 and 0.15 mg mL^{-1}). Inhibition mode was determined by double-reciprocal Lineweaver-Burk plot analysis according to the Michaelis-Menten kinetics (Zhao and Kim, 2004; Carbonell and Freire, 2005; Gholamhoseinian *et al*., 2008b).

RESULTS

Plants with HMG-CoA reductase inhibitory effect: Among one hundred extracts; *Quercus infectoria*, *Rosa damascena* and *Myrtus communis* showed 84, 70 and 62% inhibitory effect on HMG-CoA reductase activity, respectively.

Twenty one extracts showed an inhibitory effect between 30-50% on HMG-CoA reductase activity. The rest of plant extracts showed less than 30% or no inhibition on the activity of the enzyme in this study (Table 1).

Kinetic analysis of HMG-CoA reductase inhibition: The inhibition mode of *Quercus infectoria*, *Rosa damascena* and *Myrtus communis* extracts was analyzed by double-reciprocal Lineweaver-Burk plot analysis. The enzyme kinetics demonstrated non-competitive inhibition on HMG-CoA reductase activity by *Quercus infectoria* (Fig. 1), *Rosa damascena* (Fig. 2) and *Myrtus communis* (Fig. 3). The Km value of HMG-CoA for HMG-CoA reductase in the absent of the extracts was 0.6 mM and V_{max} value was 0.011 mM min^{-1}. When the extracts were added to the enzyme mixture the V_{max} value in the presence of *Quercus infectoria* extract was 0.0041, *Rosa damascena* extract was 0.0031 and for *Myrtus communis* extract was 0.0028 mM min^{-1}.

Table 1: Anti HMG-Co A reductase activity of plants extract. Reaction mixture was contained 1.2 mmol L^{-1} NADPH, 1.2 mmol L^{-1} HMG-Co A, 1U enzyme, 0.1 mol L^{-1} potassium phosphate buffer pH 7.5, 400 mM KCl, 0.1 mg mL^{-1} BSA and 3.5 mmol L^{-1} EDTA; Reduction in absorbance was assayed at 340 um

Plants name	Family	Used part	Inhibition (%)
Acantholepis orientalis	Asteraceae	Aerial parts	27.0
Achillea wilhelmsii	Asteraceae	Aerial parts	00.0
Acroptilon repeus	Asteraceae	Aerial parts	15.8
Alhagi camelorum	Fabaceae	Aerial parts	00.0
Alpinia officinarum	Zingiberaceae	Rhizomes	22.0
Althaea officinalis	Malvaceae	Flowers	00.0
Apium graveoleus	Umbelliferae	Leaves	00.0
Arctium lappa	Asteraceae	Roots	5.20
Artemisia santolina	Asteraceae	Aerial parts	00.0
Biebersteinia multifida	Berberdaceae	Aerial parts and fruits	23.3
Bryonia aspera	Cucurbitaceae	Aerial parts	20.0
Bunium persicum	Apiaceae	Seeds	29.0
Camellia sineusis	Theaceae	Leaves	47.3
Cannabis sativa	Cannabaceae	Seeds	07.4
Cardaria draba	Brassicaceae	Aerial parts and flowers	19.0
Carthamus oxyacantha	Asteraceae	aerial parts	5.30
Chaerophyllum khorassanicum	Apiaceae	Aerial parts	27.6
Cichorium intybus	Asteraceae	Roots	16.6
Ciunamomum zeylanicum	Lauraceae	Derm	13.6
Citrus anrantium	Rutaceae	Flowers	26.8
Citrus sineusis	Rutaceae	Fruits hull	22.3
Convolvulus pilosellaefolius	Concolvulaceae	Aerial parts	21.0
Cordia mixa	Boraginaceae	Fruits	27.8
Crocus sativa	Iridaceae	Leaves	00.0
Cuminum cyminum	Apiaceae	Seeds	26.0
Ducrosia asadii	Apiaceae	Aerial parts	00.0
Echium amoenum	Boraginaceae	Flowers	00.0
Eremostachys laciniata	Lmiaceae	Whole the plant	5.00
Eremurus persicus	Liliaceae	Aerial parts	15.7
Eremurus persicus	Liliaceae	Flowers	23.8
Eremurus persicus	Liliaceae	Fruits	00.0
Eucaliptus galbie	Myrtaceae	Leaves	43.0
Euphorbia hebecarpa	Euphorbiaceae	Aerial parts and flowers	27.0
Ferula assafoetida	Apiaceae	Aerial parts and flowers	20.0
Ferula oopoda	Apiaceae	Aerial parts	00.0
Ferulago angulata	Apiaceae	Aerial parts	23.8
Ficus carica	Moraceae	Leaves	20.0
Foeniculum vulgare	Apiaceae	Fruits	3.70
Francoeuria undulata	Asteraceae	Aerial parts	24.5
Fumaria parviflora	Fumariaceae	Aerial parts	08.0
Glycyrrhiza glabra	Fabaceae	Aerial parts	11.0
Gundelia tournefortii	Asteraceae	Aerial parts	40.0
Heracleum persicum	Apiaceae	Fruits	45.4
Hibiscus gossypifolius	Malvaceae	Flowers	00.0
Hyoscyamus senecionis	Solanaceae	Aerial parts and flowers	21.0
Laurus nobilis	Lauraceae	Leaves	44.4
Lawsonia inermis	Lythraceae	Leaves	40.1
Levisticum officinale	Apiaceae	Roots	48.0
Linum usitatissimum	Liliaceae	Seeds	37.0
Malva sylvestris	Malvaceae	Flowers	10.3
Marrubium anisodon	Lamiaceae	Aerial parts	00.0
Matricaria aurea	Asteraceae	Flowers	00.0
Mentha longifolia	Lamiaceae	Aerial parts	44.0
Mentha piperita	Lamiaceae	Leaves	37.3
Myrtus communis	Myrtaceae	Leaves	62.0
Nepeta crispa	Lamiaceae	Aerial parts	00.0
Nepeta saccharata	Lamiaceae	Whole the plant	29.6
Nigella sativa	Ranunculaceae	Seeds	00.0
Ocimum basilicum	Lamiaceae	Seeds	00.0
Olea europaea	Oleaceae	Leaves	37.0
Onobrychis viciifolia	Fabaceae	Aerial parts	22.2
Origanum majorana	Lamiaceae	Whole the plant	35.0

Table 1: Continued

Plants name	Family	Used part	Inhibition (%)
Otostegia persica	Lamiaceae	Aerial parts	18.0
Outreya carduiformis	Asteraceae	Aerial parts	10.5
Peganum harmala	Nitrariaceae	Aerial parts	28.5
Peucedanum aucheri	Apiaceae	Roots	37.0
Pimpinella anisum	Apiaceae	Seeds	10.5
Piper nigrum	Pipereaceae	Fruit	40.0
Pistacia vera	Anacardiaceae	Fruits hull	24.0
Punica granatum	Lythraceae	Fruits hull	21.0
Quercus infectoria	Fagaceae	Galls	84.0
Rheum ribes	Polygonaceae	Rhizomes	43.0
Rosa damascene	Rosaceae	Floret	70.0
Rosmarinus officinalis	Lamiaceae	Aerial parts	3.00
Rubia tinctorium	Rubiaceae	Roots	15.7
Salix alba	Salicaceae	Aerial parts	25.0
Salvadora persica	Salvadoraceae	Wood	31.6
Salvia rhytidea	Lamiaceae	Whole the plant	14.8
Sanguisorba minor	Rosaceae	Aerial parts	25.7
Scorphularia frigid	Scorphulariaceae	Aerial parts	13.0
Sizigium aromaticus	Caryophyllaceae	Floret	10.0
Solanum dulcamara	Solanaceae	Fruits	5.60
Sonchus asper	Asteraceae	Aerial parts	00.0
Sophora alopecuroides	Fabaceae	Aerial parts	00.0
Stachys inflate	Lmiaceae	Aerial parts	10.0
Stachys lavauduliifolia	Lamiaceae	Aerial parts	42.0
Terminalia chebulla	Combretaceae	Fruits	12.0
Tencrium polium	Lamiaceae	Aerial parts	25.0
Tencrium scordium	Lamiaceae	Aerial parts	28.8
Thymus serpyllum	Lamiaceae	Aerial parts	43.6
Trigonella foenum graecum	Fabaceae	Seeds	00.0
Urtica dioica	Urticacea	Aerial parts	31.6
Urtica ureus	Urticacea	Aerial parts	40.0
Vaccinium arcto-staphylus	Ericaceae	Fruits	26.0
Verbascum kermaneusis	Scrophulariaceae	Leaves	00.0
Verbascum songaricum	Scrophulariaceae	Aerial parts	36.8
Zataria multiflora	Lamiaceae	Aerial parts	22.0
Zhumeria majdae	Lamiaceae	Leaves	6.00
Zingiber officinale	Zingiberaceae	Rhizomes	20.0
Ziziphus spina-christi	Rhamnaceae	Leaves	17.3

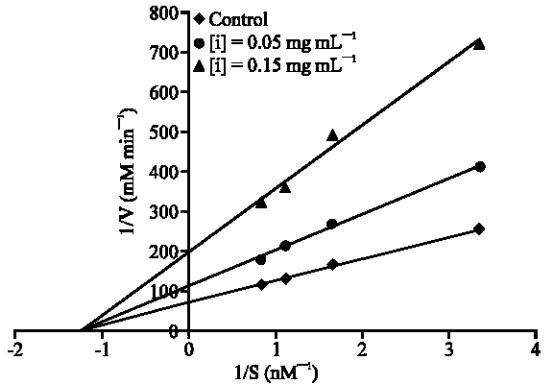

Fig. 1: The Lineweaver-Burk plot analysis for HMG-CoA reductase in presence of different concentrations of HMG-CoA (0.3, 0.6, 0.9 and 1.2 mmol L^{-1}) and two different concentrations (0.05 and 0.15 mg L^{-1}) of *Quercus infectoria* methanolic extract at 340 nm

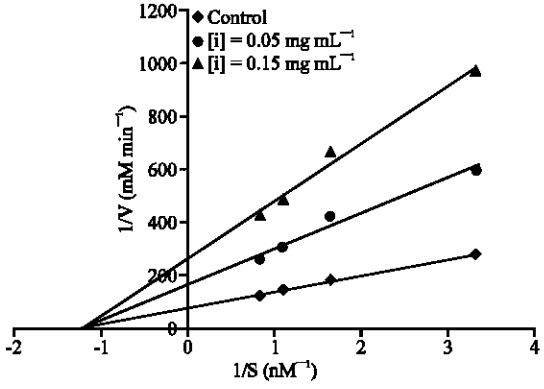

Fig. 2: The Lineweaver-Burk plot analysis for HMG-CoA reductase in presence of different concentrations of HMG-CoA (0.3, 0.6, 0.9 and 1.2 mmol L^{-1}) and two concentrations (0.05 and 0.15 mg L^{-1}) of *Rosa damascena* methanolic extract at 340 nm

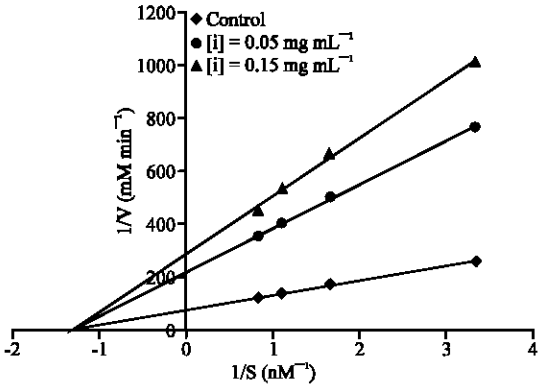

Fig. 3: The Lineweaver-Burk plot analysis for HMG-CoA reductase in presence of different concentrations of HMG-CoA (0.3, 0.6, 0.9 and 1.2 mmol L^{-1}) and two concentrations (0.05 and 0.15 mg L^{-1}) of *Myrtus communis* methanolic extract at 340 nm

DISCUSSION

The importance of plasma cholesterol level in the pathogenesis of atherosclerotic plaques has been represented by numerous studies (Yoshie *et al*., 2004). Because of various side effects of some cholesterol lowering drugs, herbal remedies have gained special attention to find the new safer compounds (Deng, 2009). Our results showed that *Quercus infectoria*, *Rosa damascena* and *Myrtus communis* are potent inhibitors of HMG-CoA reductase. Effectiveness and kinetic properties of these extracts were determined against HMG-CoA reductase which has not been showed so far. No such properties have been found for other plant extracts used in this study. The presence of HMG-CoA reductase inhibitors has been reported in some natural resources especially Kiwifruit extracts and *Vitis vinifera* (Koo *et al*., 2008; Jung *et al*., 2005). Inhibitory activities of Kiwifruit on HMG-CoA reductase activity were 14% at 10 mg mL^{-1} and 20-30% at 50 mg mL^{-1} (Jung *et al*., 2005) but our effective extracts were used at 0.15 mg mL^{-1} and showed more than 60% inhibitory effect on activity of HMG-CoA reductase which showed our plants are more potent inhibitor of HMG CoA reductase.

Yazdanparast and Bahramikia (2008) showed that *Anethum graveolens* L. crude extract has a strong hypocholesterolemic effects in rats and they showed that *Anethum graveolens* exert it's effect through inhibition of HMG CoA reductase.

It has been previously showed that *Quercus infectoria* and *Rosa damascena* have anti-porcine pancreatic lipase, anti-alpha mannosidase and anti-alpha glucosidase activities (Gholamhoseinian *et al*., 2010; 2008a, b). These are target enzymes with therapeutic potential in the treatment of hyperlipidaemia, diabetes, metastatic cancer and lysosomal storage disease. It has been also showed that *Eucaliptus galbie* and *Levisticum officinale are* potent inhibitors of lipase whereas no strong inhibitory effect was found on HMG-CoA reductase by them (Gholamhoseinian *et al*., 2010). The existence of cholesterol lowering agents have been demonstrated in different plant species including garlic (Chetty *et al*., 2003), *Morus alba*, *Melissa officinalis*, *Artemisia capillaries* (Lee *et al*., 2008), *Ananas comosus* (Xie *et al*., 2007) and *Gynostemma pentaphyllum* (Megalli *et al*., 2005) but their mechanism of cholesterol lowering effects have not been established. Probably the active ingredient (s) of these plants affects the absorption of cholesterol in the intestine. The structural similarities to cholesterol might cause the mal-absorption and excretion or affects the biosynthesis stage of cholesterol in the liver and lead to cholesterol lowering effect *in vivo* (Ros, 2000).

Quercus infectoria have great medicinal values. In Asia the galls of this plant have been used for long time in treating inflammatory diseases and possess many therapeutic activities such as antidiabetic, anti-parkinsonian, antiviral, anti- microbial, antioxidant and larvicidal properties (Aroonrerk and Kamkaen, 2009). By antioxidant and anti HMG CoA reductase activity, Quercus *infectoria* can be considered as a potent cardio-protective plant. *Rosa damascena* showed anti-diabetic effect by reducing carbohydrate absorption from the intestine (Gholamhoseinian *et al*., 2009). Other therapeutic effects of *Rosa damascena* include cardiac strengthening and anti inflammatory effect. In addition, *Rosa damascena* extract can act on central nervous system including brain and showed hypnotic effect (Rakhshandeh *et al*., 2004). *Myrtus communis* also has been employed as an antiseptic and anti inflammatory agent and in the treatment of diabetes mellitus (Yoshimura *et al*., 2008).

HMG-CoA reduction is the target enzyme for anti-cancer therapy (Wong *et al*., 2002). With this regard, plant extracts that inhibits HMG-CoA reductase may be used as cancer therapeutic agents. Statins are a group of drugs that inhibit HMG-CoA reductase in a competitive manner (Carbonell and Freire, 2005). In contrast, we showed that *Quercus infectoria*, *Rosa damascena* and *Myrtus communis* inhibit HMG-CoA reductase in a non-competitive manner. This type of inhibition

probably is dependent on especial components which bind to enzyme or enzyme-substrate complex. The anti HMG-CoA reductase activity of these plants encourages us to design an *in vivo* study on animal models to confirm their effects. It would be interesting to purify and characterize the active constituents of these extracts, to establish the composition and mechanism of action and to confirm their pharmacological potentials.

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