Iranian Journal of Pharmaceutical Sciences
Summer 2009: 5(3): 163-170
ijps.sums.ac.ir

Original Article

Inhibitory Effect of *Ruta graveolens* L. Extract on Guinea Pig Liver and Bovine Milk Xanthine Oxidase

Saeed Pirouzpanah[a], Mohammad R. Rashidi[b],*, Abbas Delazar[b], Seyed Vali Razavieh[c], Ali Asghar Hamidi[d]

[a]*Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences and Health Services, Tehran 19395-4741.*
[b]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz 51666-14776.*
[c]*Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz 51666-14776.*
[d]*School of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51666-14776, Iran.*

Abstract

Flavonoids could serve as potent inhibitors of xanthine oxidase (XO). In the present study, the effects of *Ruta graveolens* L. extract and its major isolated flavonoids, quercetin and rutin, on guinea pig liver XO have been investigated. The inhibitory effects of *R. graveolens*, quercetin and its glycoside form, rutin, were assayed spectrophotometrically. *R. graveolens* extract showed moderate inhibition on XO activity. Interestingly, bovine milk and guinea pig liver XO were inhibited significantly at different ranges by either the extract or its flavonoids, whereas allopurinol acted with almost the same potency on both enzymes. Rutin inhibited the enzymes in a competitive manner, while quercetin was found to be a competitive and mixed inhibitor of guinea pig liver and bovine milk XO, respectively. In conclusion, *R. graveolens* extract can act as a good inhibitor of XO. Interestingly, it was shown that the inhibitory effects of flavonoids on XO could be species dependent.

Keywords: Flavonoids; Quercetin; *Ruta graveolens*; Rutin; Xanthine oxidase.
Received: September 16, 2008; *Accepted:* June 11, 2009

1. Introduction

Gout and hyperurecimia are metabolic disorders resulted from abnormal production and excretion of uric acid in the body [1]. Xanthine oxidoreductase (XOR) is a cytosolic molybdenum containing enzyme mainly responsible for final reactions of uric acid production from oxidizing oxypurines, hypoxanthine and xanthine [2-5]. The XOR predominantly exists as xanthine dehydrogenase (XD, EC 1.17.1.4) in liver and intestine, but it is occasionally converted to xanthine oxidase (XO, EC 1.17.3.2) either by oxidation of SH-group of cystein residue 535 and 992 [6], reversibly or due to proteolytic reaction with Ca^{2+}-stimulated protease,

*Corresponding author: Mohammad R. Rashidi, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz 51666-14776, Iran.
Tel (+98)411-3341315; Fax (+98)411-33444280
Email: rashidi@tbzmed.ac.ir

irreversibly [7, 8]. Particularly, ischemia-reperfusion significantly predispose the conversion of XD to XO increasing the generation of hydrogen peroxide and superoxide anion which in turn can cause reperfusion injuries [7, 8]. XO also plays an important role in the metabolism of many xenobiotics and drugs such as purines and pyrimidines [9], 6-mercuptopurine and azathioprine [10, 11], thiazides [11], pyrazinamide [12], and acyclovir [13]. Therefore, the inhibition or activation of XO may result in some important therapeutic or toxic effects. Although there are many agents with XO inhibitory effect, apart from allopurinol, none of these inhibitors has implicated for clinical applications. However, allopurinol may cause some serious adverse side effects including, nephropathy, allergic reactions, severe nausea or vomiting and hepatitis [1]. Accordingly, many attempts have been made to find a suitable alternative for allopurinol [14, 15].

Certain aglycons from flavonol subtype have been shown to be potent XO inhibitors by numerous studies [15-26]. However, to date, it has not been addressed the inhibitory effect of certain flavonols on guinea pig liver XO in comparison with bovine milk XO. Guinea pig liver fraction was implied to have similarity to human liver molybdenum hydroxylase [4, 27]. *Ruta graveolens* (Rutaceae) is an herbaceous plant which is rich in flavonols and has been traditionally used owing to sedative and antihelmintic, hypotensive, antifertility, anti-inflammatory and analgesic actions [28]. The proposed therapeutic effects of *R. graveolens* have been ascribed to some chemical constituents including flavonols [29].

Recently, we have found that *R. graveolens* has a potent inhibitory effects on another molybdenum hydroxylase, aldehyde oxidase (AO, EC 1.2.3.1) [30]. The most important isolated flavonoids in *R. graveolens* were found to be rutin (quercetin-3-O-β-rutinoside) and quercetin (Figure 1). We also indicated that the effects of flavonoids on AO, which has many structural and functional similarities with XO, could be species dependent [31]. To our knowledge, there is no report on the effect of flavonoids and also the *R. graveolens* extract on XO from different sources. In the present comparative study, therefore, the effects of *R. graveolens* extract and its major flavonoids, quercetin and rutin, on XO from bovine milk (as the major source for the preparation of XO) and guinea pig liver (as one of the common laboratory animals used in the experimental studies) have been investigated.

2. Material and methods

2.1. Materials

Allopurinol, xanthine, bovine milk xanthine oxidase (grade I: from buttermilk, 0.5 unit per mg protein) were obtained from Sigma-Aldrich (Poole, Dorset, England). All other chemicals, apart from quercetin and rutin, were purchased from Merck (Darmstadt, Germany).

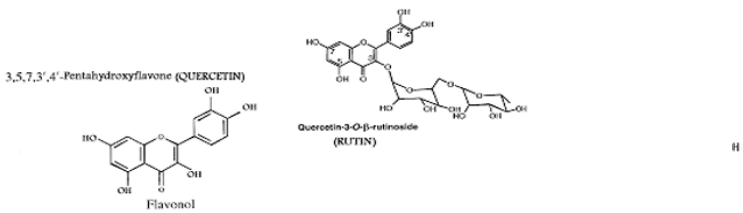

Figure 1. Chemical structures of rutin and quercetin.

Table 1. IC_{50} values and inhibitory activity of *R. graveolens* extract, quercetin, rutin and allopurinol on uric acid production progress by guinea pig liver and bovine milk XO[a].

Inhibitors	Bovine milk XO			Hepatic guinea pig XO	
	Conc.[b]	% Inhibition[c]	IC_{50}[b]	Inhibition	IC_{50}
R. graveolens extract	100	35.6±4.5[c]	110±43	59.6±9.3	87.5±2.1
	50	17.6±5.7		35.2±11.1	
	10	15.5±0.1		17.5±10.6	
Quercetin	10	95.1±2.6	0.4±0.01	98.2±7.6	0.7±0.1
	1	82.4±0.7		67.3±8.3	
Rutin	100	67.2±4.1	39.4±15.8	71.3±2.4	15.8±2.1
	10	21.8±8.3		43.8±6.7	
	1	9.3±1.4		26.5±7.1	
Allopurinol	100	94.2±1.4	2.9±0.9	97.1±6.2	2.1±0.8
	10	75.3±8.7		81.5±9.6	
	1	44.1±11.7		46.7±12.3	

[a]The initial velocity for the oxidation of xanthine due to XO from bovine milk and guinea pig liver are 6.4±0.3 and 2.6±0.2 nM/min./mg protein (n=6). Xanthine was used in 5×10^{-5} Mole as substrate. [b]The units of concentration and IC_{50} values for the extract have been expressed in µg/ml and for allopurinol, quercetin and rutin in µM. [c]The results are expressed as percentage inhibition (mean±SD, n=6).

2.2. Plant material

Leaves of *R. graveolens* were harvested from garden maintained in July 2007 at Tabriz University of Medical Science, Tabriz, Iran. A voucher specimen was deposited with PRTRG001, and a representative of this collection has been retained in the herbarium of the Pharmacognosy Department, School of Pharmacy, Tabriz University of Medical Sciences. Leaves of the plant were dried at room temperature for two weeks and ground using an electric mill.

2.3. Extraction preparation and isolation and structure identification of quercetin and rutin

The preparation procedure of total extract with more details was reported in our previous reprot [30]. In summary, the dried, ground leaves of *R. graveolens* (100 g) were extracted with 70% methanol at room temperature followed by rotatory evaporation in low pressure yielding the dried extract (12.65 g). The dried material was suspended in distilled water, and extracted with petroleum ether, chloroform, ethyl acetate and n-butanol at a maximum temperature of 45 °C to obtain the respective fractions. The ethyl acetate fraction was subjected to column chromatography on

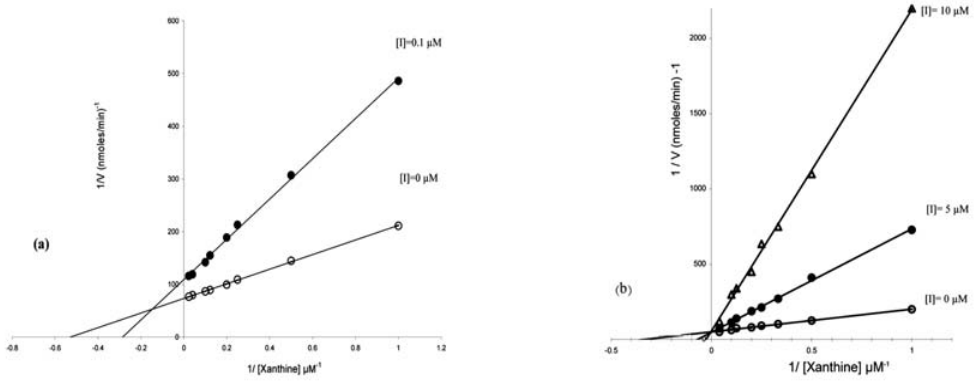

Figure 2. Typical Linweaver-Burk plots for the oxidation of xanthine by bovine milk (a) and guinea pig liver XO (b) in the presence of quercetin.

Table 2. Kinetic characteristics of inhibitory activity of quercetin and rutin on the oxidation of xanthine by guinea pig liver and bovine milk XO[a].

Inhibitors	Bovine milk XO			Hepatic guinea pig XO		
	Ki (μM)	KI (μM)	Mode	Ki (μM)	KI (μM)	Mode
Quercetin	0.06±0.03[b]	0.22±0.16	Mixed	0.11±0.02	-	Competitive
Rutin	0.70±0.10	-	Competitive	3.01±0.12	-	Competitive

[a]The Km and Vmax values for bovine milk and hepatic xanthine oxidase were calculated as follows: 2.9±0.4 μM, 27.4±4.8 μM/min./mg protein and 6.1±2.4 μM, 35.7±3.4 μM/min./mg protein, respectively. [b]The results are expressed as Mean±S.D, n=3.

silica gel using ethyl acetate-methanol-water (70:15:5 v/v/v) as the mobile phase. The flow rate of the organic phase was maintained at 6 drops per min. TLC of column chromatography fractions was carried out on silica gel plates using the mobile phase consisting of ethyl acetate-methanol-water (65:10:15 v/v/v). The main components of the fractions of 55-70 were further purified by preparative TLC on silica gel with a mobile phase of ethyl acetate-methanol (90:10 v/v) which resulted in the isolation of 14.6 mg quercetin. The n-butanol extract was fractioned on a Sep-Pak using a step gradient of 10, 20, 40, 60, 80 and 100% methanol-water mixture (150 ml each) as the eluent. Preparative RP-HPLC (Shim-Pak CLC-C18, 6-150 mm i.d.); gradient elution, 25-70% methanol in water in 50 min. (20 ml/min.) of the Sep- Pak fraction (60% methanol in water) yielded 82.8 mg rutin. The structures of the both compounds were determined conclusively by UV spectroscopy, ^{1}H- and ^{13}C-NMR analysis [32, 33].

2.4. Preparation of partially purified guinea pig hepatic XO

Partially purified XO were prepared from mature male Dunkin-Hartley guinea pig liver (400-600 g, Tabriz University of Medical Sciences, Tabriz, Iran) according to the method of Johnson *et al*. [34]. Briefly, the animal was killed by cervical dislocation. Then, the liver was immediately excised and placed in ice-cold isotonic potassium chloride solution (1.15% KCl w/v) containing 0.1 mM EDTA. The liver was homogenized at 3000-4000×g for 1 min. at 4 °C using Potters homogenizer. The homogenate was heated on a steam bath at 55-57 °C, then cooled to 4 °C and centrifuged at 15000×g for 45 min. The supernatant was treated by 50% saturated solution of ammonium sulphate (35.3 g/100 ml) at 4 °C. The resulting suspension was re-centrifuged at 6000×g for 20 min. at 4 °C. The precipitate was dissolved in a minimum volume of 0.1 mM EDTA solution and ultimately they were kept at -70 °C till the time of examinations.

2.5. XO assays

All spectrophotometric determinations were carried out at 37 °C using a Shimadzu UV-visible spectrophotometer UV-2550, which was controlled by the Shimadzu UV Probe personal software package including kinetics software. The instrument was connected to a Shimadzu cell temperature regulatory unit to keep the temperature at 37 °C, constantly. The cuvette used had a path length of 1 cm and the total reaction mixture volume was constant at 3.0 ml. XO activity was assayed by monitoring of uric acid production at 295 nm. Xanthine (50 μM), as specific substrate for XO, was incubated with the enzyme fraction in Sorenson's phosphate buffer (67 mM, pH 7.0) containing 0.1 mM EDTA and the initial oxidation rates were measured up to 3 min. The reactions were also measured in the presence of different concentrations of *R. graveolens* extract, quercetin and rutin to assay the inhibitory effects. The results were compared with the inhibitory effects of allopurinol, the potent specific inhibitor of XO [3, 23].

2.6. Determination of kinetic constants

The IC_{50} values of *R. graveolens* extract, quercetin and rutin were obtained from the

inhibitor concentration-activity curve. Michaelis-Menten constant (Km) and maximum initial velocity (Vmax) values for the oxidation of xanthine by the enzyme fractions were determined spectrophotometrically from a Lineweaver-Burke double reciprocal plot of 1/v against 1/[xanthine]. The best fit line through the plotted points was calculated using linear regression by the least square method. The reactions were also studied in the presence of quercetin and rutin. The inhibition constants were determined using secondary plot (slopes and intercepts from the initial Lineweaver-Burk plot *vs* inhibitor concentrations). In the case of mixed inhibition where inhibitor can bind to the free enzyme and to the enzyme-substrate complex, two inhibitor constants were defined: K_i as the dissociation constant of the enzyme-inhibitor complex, and K_I as the dissociation constant of the enzyme-substrate-inhibitor complex. If $Ki<K_I$, the inhibition was considered as a competitive-noncompetitive type of the inhibition; if $Ki>K_I$, the inhibition was considered as uncompetitive-noncompetitive type [35].

2.7. Protein determination

Protein concentrations of partially purified enzyme fractions were determined spectrophotometrically using a Pierce BCA Protein assay kit with bovine serum albumin as a protein standard [36].

3. Results

The ^{1}H-NMR and ^{13}C-NMR of the isolated compounds from the extract significantly revealed the chemical shifts of protons and carbons, which is consistent with those reported in the literature for quercetin and rutin [30, 32]. The chemical structures of quercetin and rutin also have been confirmed by analysis of the UV spectra of the compounds with the diagnostic shift reagents [33].

In Table 1, the inhibition of XO by *R. graveolens* extract and its major flavonoids, quercetin and rutin, in comparison with the inhibitory effect of allopurinol, a potent inhibitor of XO, have been tabulated. The *R. graveolens* extract inhibited bovine milk XO activity by 37% at 100 mg/ml with an IC_{50} value of 110 µM. The corresponding values for the inhibition of the guinea pig liver enzyme were 60% and 88 µM, respectively. Bovine milk and guinea pig liver XO were inhibited significantly with 10 µM quercetin (95.1 and 98.2%) and estimated IC_{50} values were 0.4 and 0.7 µM, respectively. Rutin caused weak inhibition on uric acid production by both enzymes (21.8 and 43.8%) at 10 µM with IC_{50} values of 39.4 and 15.8 µM, correspondingly. The inhibitory activities of allopurinol against both enzymes were determined at IC_{50} values of 2.9 and 2.1 µM, respectively.

The inhibitory features induced by quercetin and rutin, on bovine milk and hepatic XO were further investigated by the enzymatic kinetic parameters (Table 2). Quercetin inhibited the bovine milk XO in a mixed manner (competitive-noncompetitive type) with kinetic constants of K_i=0.06±0.03 and K_I=0.22±0.16 µM (Figure 2a). This flavonol inhibited competitively the guinea pig hepatic enzyme at Ki=0.11±0.02 (Figure 2b). Rutin applied its inhibitory effects on the bovine milk and guinea pig liver XO through competitive inhibitory manner with kinetic constants of 0.70±0.10 and 3.01±0.12, respectively (Table 2).

4. Discussion

XO contributes to metabolic clearance of endogenous purines, aldehydes and also some important xenobiotics [3]. Additionally, it has been shown that the activity of this enzyme is commonly over-expressed in ischemia-reperfusion injury and some other related clinical complications such as brain tumor, hepatitis, organ transplantations, birth trauma, and sever intense physical activity [7, 37]. Accordingly, there is a keen interest to find an efficient XO inhibitor as a therapeutic agent particularly from natural sources.

Medicinal plants are one of the major options for finding and developing new remedies [1, 38].

There is a large body of evidence indicating that some flavonoids are potent inhibitors of XO, particularly on bovine milk XO [15-26]. *R. graveolens* is an herbaceous plant, which is rich in flavonoids as its major active reagents [28]. More recently, it has been shown that the extract of this medicinal plant can act as an inhibitor of AO, another molybdenum hydroxylase [30]. With this regard, the effect of *R. graveolens* extract on XO activity was investigated and provided evidences indicated that the extract was able to exhibit moderate inhibitions on bovine milk and guinea pig liver XO activities. The inhibitory effect of quercetin on either type of enzyme was found to be relatively higher than that observed with allopurinol, which is consistent with those results reported by the others [18, 23].

There was also a marked potent inhibitory effect of quercetin on XO compared with that of rutin as it has been pronounced by others [23-25]. It is well documented that the bulky glycosyl group substitution on C-3 of quercetin attenuates significantly the binding affinity of the glycosidic flavonoid with the enzyme [16, 23]. The importance of hydroxyl group at C-3 position in C ring of the flavonoid has also been demonstrated for AO. Likewise their effects on XO, guinea pig liver AO is inhibited more markedly by quercetin than rutin [30, 31]. Similar structural concept has also been reported for the inhibition of AO by steroids. The substitution of hydroxyl group at C-3 of A ring of β-estradiol proposed to reduce the inhibitory effect of steroid on AO activity [39].

Interestingly, quercetin and rutin displayed different degrees of inhibition on bovine milk XO versus guinea pig liver XO. In this case, the aglycone (quercetin) caused a potent inhibition on the former enzyme, whereas rutin exert effective inhibition against guinea pig liver XO rather than bovine milk XO (Table 1). However, this different inhibitory activity was not observed, while allopurinol was employed. Indeed, unlike allopurinol, theses flavonoids impart with different mode of inhibition on bovine milk and guinea pig liver XO activities. The enzymatic kinetic parameters indicated that quercetin inhibited bovine milk XO in a mixed manner, while the guinea pig hepatic enzyme was inhibited competitively by this flavonoid. It is possible that bovine milk XO compared with the guinea pig liver enzyme has an extra site in addition to the active site for binding of quercetin, which results more influential inhibition on bovine milk XO (smaller IC_{50} value) than the guinea pig hepatic enzyme. Rutin exerted its inhibitory effect on the bovine milk and guinea pig liver through competitive inhibitions. Although the provided evidences on XO and AO show species variation (the substrate specificity and inhibitory kinetic properties), the XO was shown to be less species- and tissue-variant than the AO [2-4].

Even though it can be concluded that the observed different inhibitory behaviors of the studied flavonoids may associate with the species variations, the chemical structure of inhibitors may also have considerable impact. Hence, the subject of species variation may be clarified by performing further studies with more flavonoids.

5. Conclusion

It was shown that *R. graveolens* with high flavonol-content can serve as a good inhibitor of XO. Whether this natural plant can be administered as an alternative to allopurinol, is subjected for further studies. The data reported in the present study indicate that the inhibitory effects of flavonoids on XO could be species dependent. As these natural flavonoids are found ubiquitously in many vegetables, fruits and plant-derived remedies, their effects on XO have received much

attention from researchers, and it would be of value to perform more studies to find the best experimental animal model for human XO/XD system with respect to these polyphenols.

Acknowledgements

We would like to thank Drug Applied Research Center, Tabriz University of Medical Sciences for their financial support.

References

[1] Kong D, Zhang Y, Pan X, Tan R, Cheng C. Inhibition of xanthine oxidase by liquiritigenin and isoliquiritigenin isolated from *Sinofranchetia chinesis*. *Cell Mol Life Sci* 2000; 57: 500-5.

[2] Beedham C. Molybdenum hydroxylases. In: Gorrod J, Oeschlager H, Caldwell J, (editors). *Metabolism of xenobiotics*. London: Taylor & Francis, 1998; pp. 51-8.

[3] Beedham C, Molybdenum hydroxylases. In: Ioannides C, (editor). *Enzyme systems that metabolise drugs and other xenobiotics*. Chichester: Wiley & Sons Ltd, 2002; pp. 148-77.

[4] Beedham C, Bruce S, Critchely D, Al-Tayib Y, Rance D. Species variation in hepatic aldehyde oxidase activity. *Eur J Drug Metab Pharmacokinet* 1987; 12: 307-10.

[5] Linder N, Martelin E, Lapatto R, Raivio K. Post-translational inactivation of human xanthine oxidoreductase by oxygen under standard cell culture conditions. *Am J Physiol Cell Physiol* 2003; 285: C48-55.

[6] Maia L, Mira L. Xanthine oxidase and aldehyde oxidase: A simple procedure for the simultaneous purification from rat liver. *Arch Biochem Biophys* 2002; 400: 48-53.

[7] Halliwell B, Cutteridge M. *Free radicals in biology and medicine*, New York: Oxford Uiversity Press, 1999.

[8] Saksela M, Lapatto R, Raivio K. Irreversible conversion of xanthine dehydrogenase into xanthine oxidase by a mitochondrial protease. *FEBS Lett* 1999; 443: 117-21.

[9] Krenitsky T, Spector T, Hall W. Xanthine oxidase from human liver: Purification and characterization. *Arch Biochem Biophys* 1986; 247: 108-19.

[10] Van Scoik K, Johnson C, Porter W. The pharmacology and metabolism of the thiopurine drugs 6-mercaptopurine and azathioprine. *Drug Metab Rev* 1985; 16: 157-74.

[11] Tapner M, Jones B, Wu W, Farrell G. Toxicity of low dose azathioprine and 6-mercaptopurine in rat hepatocytes. Roles of xanthine oxidase and mitochondrial injury. *J Hepatol* 2004; 40: 454-63.

[12] Yamamoto T, Moriwaki Y, Suda M, Nasako Y, Takahashi S, Hiroishi K, Nakano T, Hada T, Higashino K. Effect of BOF-4272 on the oxidation of allopurinol and pyrazinamide *in vivo*. Is xanthine dehydrogenase or aldehyde oxidase more important in oxidizing both allopurinol and pyrazinamide. *Biochem Pharmacol* 1993; 46: 2277-84.

[13] Krenitsky T, Hall W, De Miranda P, Beauchamp L, Schaeffer H, Whiteman P. 6-Deoxyacyclovir: A xanthine oxidase-activated prodrug of acyclovir. *Proc Natl Acad Sci USA* 1984; 81: 3209-13.

[14] Cotelle N. Role of flavonoids in oxidative stress. *Current Topics Med Chem* 2001; 1: 569-90.

[15] Kitamura S, Sugihara K, Ohta S. Drug-metabolizing ability of molybdenum hydroxylases. *Drug Metab Pharmacokinet* 2006; 21: 83-98.

[16] Lin C, Chen S, Chen C, Liang Y, Lin J. Molecular modeling of flavonoids that inhibits xanthine oxidase. *Biochem Biophys Res Commun* 2002; 294: 167-72.

[17] Jiao R, Ge H, Shi D H, Tan R. An apigenin-derived xanthine oxidase inhibitor from *Palhinhaea cernua*. *J Nat Prod* 2006; 69: 1089-91.

[18] Aucamp J, Gaspar A, Hara Y. Inhibition of xanthine oxidase by catechins from tea (*Camellia sinesis*). *Anticancer Res* 1997; 17: 4381-6.

[19] Owen P, Johns T. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. *J Ethnopharmacol* 1999; 64: 149-60.

[20] Cos P, Ying L, Calomme M, Hu J, Cimanga K, Van Poel B, Pieters L, Vlietinck A, Vanden Berghe D. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J Nat Prod* 1998; 61: 71-6.

[21] Middleton J, Chithan K, Theoharides C. The effect of flavonoids on mammalian cells: Implication for inflammation, heart disease and cancer. *Pharmacol Rev* 2000; 52: 673-751.

[22] Selloum L, Reichl S, Muller M, Sebihi L, Arnhold J. Effect of flavonols on the generation of superoxide anions radicals by xanthine oxidase stimulated neutrophils. *Arch Biochem Biophys* 2001; 395: 49-56.

[23] Van Hoorn E, Nijveldt R, Van Leeuwen P, Hofman Z, Rabet L, De Bont D, Van Norren K. Accurate prediction of xanthine oxidase inhibition based on the structure of flavonoids. *Eur J Pharmacol* 2002; 451: 111-8.

[24] Zhu J, Wang Y, Kong L, Yang C, Zhang X. Effects

of *Biota orientalis* extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J Ethnopharmacol* 2004; 93: 133-40.

[25] Nagao A, Seki M, Kobayashi H. Inhibition of xanthine oxidase by flavonoids. *Biosci Biotechnol Biochem* 1999; 63: 1787-90.

[26] Nijveldt R, Van Nood E, Van Hoorn E, Boelens P, Van Norren K, Van Leeuwen P. Flavonoids: A review of probable mechanisms of action and potential application. *Am J Clin Nutr* 2001; 74: 418-25.

[27] Beedham C, Critchley D, Rance D. Substrate specificity of human liver aldehyde oxidase toward substituted quinazolines and phthalazines: A comparison with hepatic enzyme from guinea pig, rabbit, and baboon. *Arch Biochem Biophys* 1995; 319: 481-90.

[28] Skidmore-Roth L., *Mosby's handbook of herbs and natural supplements*. St. Louis: Mosby, 2001.

[29] Duke J, Bogenschutz- Godwin M, Du Cellier J, Duke P, *Handbook of medicinal herbs*. Boca Raton: CRC press, 2002.

[30] Pirouzpanah S, Rashidi M, Delazar A, Razavieh S, Hamidi A. Inhibitory effects of *Ruta graveolens* L. extract on guinea pig liver aldehyde oxidase. *Chem Pharm Bull* 2006; 54: 9-13.

[31] Pirouzpanah S, Hanaee J, Razavieh S, Rashidi M. Inhibitory effects of flavonoids on aldehyde oxidase activity. *J Enzyme Inhib Med Chem* 2008; 24: 14-21.

[32] Breitmaier E, Voelter W., *Carbon- 13 NMR spectroscopy*. New York: VCH Verlagsgesellschaft mbH, 1989.

[33] Mabry T, Markham K, Thomas M, *The systematic identification of flavonoids*. New York: Spring-Verlag, 1970.

[34] Johnson C, Stubley-Beedham C, Stell J. Hydralazine: A potent inhibitor of aldehyde oxidase activity *in vitro* and *in vivo*. *Biochem Pharmacol* 1985; 15: 4251-6.

[35] Marangoni A, Enzyme kinetics, a modern approach. New Jersey: John Wiley, 2003.

[36] Smith P, Krohn R, Hermanson G, Mallia A, Gartner F, Provenzano M, Fujimoto E, Goeke N, Olson B, Klenk D. Measurement of protein using bicinchoninic acid. *Anal Biochem* 1985; 150: 76-85.

[37] Judge A, Dodd S. Xanthine oxidase and activated neutrophils cause oxidative damage to skeletal muscle after contractile claudication. *Am J Physiol Heart Circ Physiol* 2004; 286: H252-6.

[38] Dew T, Day A, Morgan R. Xanthine oxidase activity *in vitro*: Effects of food extracts and components. *J Agric Food Chem* 2005; 53: 6510-5.

[39] Obach R. Potent inhibition of human liver aldehyde oxidase by raloxifene. *Drug Metab Dispos* 2004; 32: 89-97.