Tropical Journal of Pharmaceutical Research June 2014; 13 (6): 951-956
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i6.18

Original Research Article

Total Antioxidant Activity, and Hesperidin, Diosmin, Eriocitrin and Quercetin Contents of Various Lemon Juices

Mannan Hajimahmoodi[1]*, Ghazaleh Moghaddam[1], Seyed Mohsen Mousavi[1], Naficeh Sadeghi[1], Mohammad Reza Oveisi[1] and Behrooz Jannat[2]
[1]Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, [2]Drug and Food Control Laboratory, Research Center, Ministry of Health and Medical Education, Tehran, Iran

For correspondence: **Email: hajimah@sina.tums.ac.ir; **Tel/Fax:** +98-21- 66954714*

Received: 12 June 2013 Revised accepted: 27 March 2014

Abstract

Purpose: *To investigate 38 natural and 62 branded lemon juices for their total antioxidant activities and flavonoid content.*
Methods: *The ferric reducing antioxidant power was applied to evaluation of antioxidant. Additionally quercetin, eriocitrin, diosmin and hesperidin contents were evaluated by high performance liquid chromatography (HPLC).*
Results: *Total antioxidant activity of the juices ranged from 91.38 ± 0.01 to 526.93 ± 0.12 μg/ml. The maximum contents of quercetin, hesperidin and eriocitrin 14.83 ± 2.83, 104.84 ± 12.58 and 10.68 ± 2.41 μg/mL, respectively. The natural juices had diosmin levels as high as 236.18 mL. The limit of detection (LOD) ranged from 0.15 μg mg-1 for hesperidine to 0.53 μg ml-1 for diosmin while the limit of quantification (LOQ) ranged from 0.27 μg mg-1 for hesperidine to 0.93 μg ml-1 for quercetin.*
Conclusion: *The developed method can also be applied to the determination of related extracts of orange cultivars as well as various extracts of medicinal plants.*

Keywords: *Antioxidant, Flavonoid, Hesperidin, Diosmin, Eriocitrin, Quercetin, Lemon juice*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Lemon (*Citrus limon* L) is the third most important species of citrus fruit after orange and mandarin. The presence of bioactive compounds, such as hydrocinnamic acid, ferulic acid, cyaniding glucoside, flavonoid, vitamin C, carotenoid, hesperidin and naringin content contribute to the value of lemon in terms of it being associated with promoting good health [1]. Phenolic and flavonoids compounds are crucial for plants growth and reproduction, and are produced as a response to environmental factors and to defend injured plants [2-4].

Recently, more attention had been paid to plant flavonoids and some publications have suggested they might play important roles in anticancer activity [5-7]. An imbalance between antioxidants and free radicals results in oxidative stress, will lead to cellular damage [8]. Quercetin, as a flavonol, is used as a nutritional supplement found in fruits and vegetables. It is thought to have potent antioxidant, ant diabetic and anti tumor, and antiviral, anti inflammatory benefits [9,10]. Eriocitrin is an ingredient derived from natural products, and it is contained in a juice of citrus fruits. Another determined flavonoid is a synthetic antioxidant called diosmin. Recent clinical studies have demonstrated that the diosmin can be used to treat venous leg ulcers and hemorrhoids. Diosmin drugs have been successfully used as chemo-preventive agents in urinary-bladder 9 and colon carcinogenesis [11]. Hesperidin is a flavanone glycoside found abundantly in citrus fruits which is believed to play a role in plant defense and it acts as an antioxidant according to *in vitro* studies [12].

The purpose of this work was to determine the total antioxidant activities of several lemon juices by a spectrophotometric method as well as determine the flavonoid (quercetin, hesperidin, diosmin, and eriocitrin) contents of the juices by HPLC for the purpose of developing a suitable quality control for levels of these compounds in plant materials.

EXPERIMENTAL

Chemicals, reagents and materials

All solvents/chemicals used were of analytical grade and were purchased from Merck Company (Darmstadt, Germany). Quercetin, eriocitrin, diosmin and hesperidin (Sigma, St. Louis) were used as standards.

Sample preparation

The lemon juice samples were divided into two groups (38 natural lemon juices from local sources and 62 branded lemon juices from supermarkets). Lemons in the first group were washed, peeled and squeezed to extract the juice and then clarified with Whatman no. 4 filter paper. All samples were stored as recommended on the labels and were analyzed before expiry dates. This study was done during the fall and winter months of 2012.

Total antioxidant activity

FRAP (ferric reducing antioxidant power) assay is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous blue colored form in the presence of antioxidants. It is a relatively simple method frequently used in the assessment of antioxidant activity of various fruits, vegetables and some biological samples [13].

Briefly, FRAP reagent contained 5 ml of 2, 4, 6-tripyridyl-s-triazine (TPTZ, 10 mmol/L) solution in HCl (40 mmol/L) plus 5 mL of $FeCl_3$ (20 mmol/l) and 50 mL of acetate buffer (0.3 mol/L, pH 3.6). It was freshly prepared and warmed up to 37 ºC. A volume of 100 µL samples (filtered lemon juice) was mixed with 3 ml of FRAP reagent and the absorbance of the reaction mixture was spectrophotometrically measured at 593 nm after incubation at 37 ºC for 10 min. To construct calibration curve, five concentrations of $FeSO_4$ $7H_2O$ (1000, 750, 500, 250, 125 µmol/lit) were used and the absorbance were measured as sample solution. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$. All the measurements were taken in triplicate and the mean value ± standard deviation (SD) were reported.

Chromatographic conditions

These experiments were done using an analytical HPLC system consisting of a pump (Maxi-Star K-1000, Knauer, Germany), a UV spectrophotometer detector (Knauer, Germany), controlled by software (EuroChrom 2000, Version 1.6, Knauer Co., Germany). Chromatographic analysis was carried out by Eurospher 100 C8 Column (4.6 mm × 25 cm, 5 µm; Knauer, Germany) reversed phase column. The mobile phase was water; acetonitrile: acetic acid (77:21:2 v/v/v). The mobile phase was filtered through a 0.45 µm membrane filter and degassed ultrasonically prior to use. RP-HPLC separation of standard quercetin, eriocitrin, diosmin and hesperidin was set at 280 nm. Flow rate and injection volume were 1 ml /min and 10 µl. The freshly squeezed juice was centrifuged at 3000 rpm for 10 min. The pH of juice was adjusted at 8.5 by NaOH (10 %) then it was filtered through a specify membrane material of the 0.45 µm filter to analyze by HPLC. The chromatographic peaks of the analytes were confirmed by comparing their retention time with those of the reference standards. All chromatographic operations were carried out at ambient temperature.

Method validation

To check the accuracy of the method, natural samples were spiked with the flavonoids of interest at intermediate concentrations of the each calibration curves. The concentrations were recalculated from the corresponding calibration curve (experimental concentration) and compared with the theoretical concentrations. Recovery was determined as the relationship between the experimental and the theoretical concentration expressed as a percentage (C exptal/C theoretical) × 100.

Statistical analysis

All analyses were done in triplicate (n=3). The results are reported as mean ± standard deviation. Statistical analysis was carried out using t SPSS 17.0 software (SPSS Inc, Chicago, USA) and comparison of averages was based on the analysis of variance (one-way ANOVA) at a significance level of $p < 0.05$.

RESULTS

Ferric reducing antioxidant power (FRAP)

Table 1 presents the validation data of four groups of samples. The recoveries (n=6) ranged from 89.6 ± 0.9 % in diosmin to 95.0 ± 0.7 % in quercetin. Statistical analysis showed good standard deviations and recovery values.

The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to that of 1 μM/L $FeSO_4$ (Y = 0.01X-0.008, R^2 = 0.997). Most of the samples showed considerable amount of antioxidant. The antioxidant potential of studied samples were in the increasing order of no. 3 > no. 1 > Natural > no. 4 > no. 2 (Table 2).

Flavonoid analysis of the samples by HPLC

The results obtained from the preliminary analysis of flavonoids are shown in Table 1.

Quercetin content in natural sample (4.04 ± 0.79 μg/mL) was found to be highest compared to other samples. In contrast nos. 3 and 4 lacked quercetin content. Quercetin belongs to the flavonoid group with powerful antioxidant activity. It can be seen from the data in Table 1 that hesperidin content in natural samples (67.11 μg/mL) and no. 2 (49.06 μg/mL) are moderate, when compared with no. 1 (104.84 ± 12.58). Diosmin and eriocitrin were not detected in sample nos. 3 and 4 and the maximum content of diosmin that was detected was in natural group with concentration of 236.18 ± 31.70 μg/mL while, it was not observed in nos. 3 and 4. The eriocitrin content in no. 1 (10.68 μg/mL) was significantly more than others ($p < 0.05$).

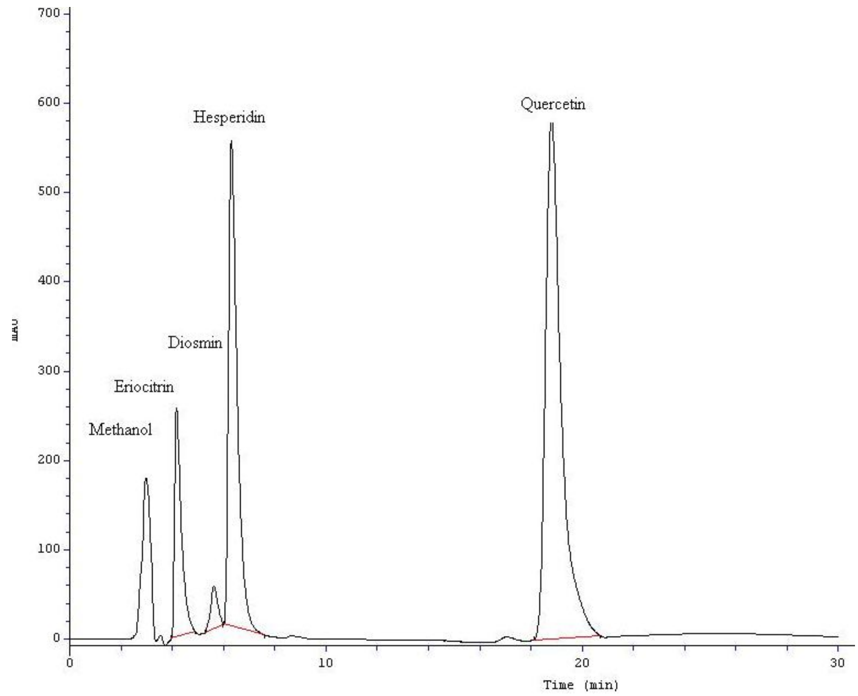

Figure 1: The mix of flavonoid compounds found in the standard solution (10 μg/mL)

Table 1: LOD, LOQ and recoveries (μg ml^{-1}) of the flavonoid in lemon juice samples

Parameter	Flavonoid			
	Quercetin	Eriocitrin	Diosmin	Hesperidin
Spiked level	10	20	15	35
Recovery (%, n=6)	95.0±0.73	92.9±0.67	89.6±0.95	91.5±0.52
LOD	0.40	0.24	0.53	0.15
LOQ	0.93	0.41	0.66	0.27
Calibration equation	Y=3.85X+27.14	Y=4.144X9.55	Y=0.241X+5.6	Y=4.489X+21.74
R^2	0.955	0985	0.964	0.988
Linear rang (μg ml^{-1})	5-25	10-30	10-25	25-60

Table 2: The antioxidant activities and the results obtained from the analysis of flavonoids in studied samples (μg/mL)

Sample	Number	Eriocitrin	Diosmin	Hesperidin	Quercetin	Total antioxidant
Natural	38	6.86±5.80	236.18±31.70	67.11±15.55	4.04±0.79	240.43±0.06
1	22	10.68±2.41	173.37±57.13	104.84±12.58	14.83±2.83	402.81±0.05
2	12	3.24±1.23	44.39±3.92	49.06±11.96	0.36±0.06	91.38±0.01
3	17	ND	ND	3.24±0.28	ND	526.93±0.12
4	11	ND	ND	8.95±2.22	ND	119.03±0.01
Range	-	0-24.02	0-478.56	5.99-129.11	0-40.61	29.58-660.13

ND = not detected ($p < 0.05$)

DISCUSSION

Ferric reducing antioxidant power assay was used to evaluate the antioxidant potential of different brands of citrus lemon juice. Principally, FRAP assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction [14]. The higher absorbance was related to the high FRAP value. The phenolic compounds exhibit extensive free radical scavenging activities through their reactivity as hydrogen or electron-donating agents and metal ion chelating properties [15].

The results of a previous study revealed that total phenolic contents in both groups were not significantly different but the group of Natural lemon juice samples had the higher antioxidant capacity and higher ascorbic acid content [1].

Based on earlier work done by Jayaprakasha *et al* [16], the antioxidant activities of citrus species are in accordance with their amount of phenolics. Therefore, nos. 2 and 4 contained low phenolic content compared to other samples, which was responsible for its low antioxidant activity. A close relationship between total phenolic content and high antioxidant activity had previously been established by the numerous reports [17-19].

The antioxidant activity of lemon samples (*Citrus limon* L) reported by Ali *et al* [20] was 42.31 ± 0.52 mg of Trolox equivalent/100 g of fruit, while Amla (*Emblica officinalis*) sample showed activity of 1577 ± 4.56 mg of Trolox equivalent/100 g of fruit. Thus, citrus fruits exhibit intermediate antioxidant activity in the study, with sweet lime as the most effective followed by orange, pummel, lemon etc. In China, the highest content of ascorbic acid equivalent antioxidant capacity was related to Hamlin (899.31 mg/L) among fifteen citrus varieties which had been determined by FRAP assay [21].

According to Xu *et al* [22], total phenolics (gallic acid equivalent), FRAP, DPPH, total flavanone glycosides and total phenolic acids indicate phenolics plays an important role, which may be mainly ascribed to flavanone glycosides, whilst phenolic acids seemed to play a minimal role [22].

Previous studies showed that quercetin may help to prevent cancer, especially prostate cancer. Scambia [23] reported quercetin inhibited human breast cancer cells (MCF–7 and MDA–MB–231) significantly.

In Wu *et al*, the detected hesperidin was 17.86 (μg/mL) in grapefruit juice and 714 (μg/mL) in grapefruit peel [24]. As a comparison with other fruit juices, the average amount of hesperidin in lemon, grapefruit, grapefruit white and grapefruit red-pink are 15.78, 2.78, 3.95 and 0.27 mg/100g fresh respectively [25].

Diosmin has certain biological activities, including anti-inflammatory effect and inhibition of prostaglandin synthesis [26]. Various psychological functions have attributed to eriocitrin of fruits. The determined content of eriocitrin in lemon, grapefruit, grapefruit white and grapefruit red-pink are, 9.46, 0.45, 0.16 and 0 mg/100g fresh juice in Peterson *et al* [25]. In another study, the measured eriocitrin level was 1.54 μg/mL in peel and 216 μg/mL in juice of *Citrus limon* and it didn`t detected in grapefruit, kinkan, buntan and kabosu [17].

In Fadlinizal Abd Ghafar *et al* [27], the flavonoid content of some citrus lemon juice species were expressed. The high amount of hesperidin was found in Wild lime (*C. hystrix*) by 22.25 ± 0.20 mg of hesperidin equivalent / 100 mL of juice and in Common lime (*C. aurantifolia*) by 16.67 ± 2.57 mg hesperidin / 100 mL of juice. The flavonoid content of citrus species varied from 22.25 ± 0.20 in *C. hystrix* to 2.99 ± 0.09 mg hesperidin equivalent/100 mL of juice in *C. sinensis* [27]. Also hesperidin (6.89 ± 0.06 to 26.98 ± 0.07 mg/g) was present at high concentrations compared to the other flavonoid in the pulp extracts [28]. Among fifteen citrus varieties, Yinzaocheng had the highest content of hesperidin (533.64 mg/L) in Xu *et al* [22]. It should be noted that fruit peel is sometimes mixed with juice in the commercial production of fruit juice and this may be the main cause for the high amounts of total phenolic content in some commercial branded lemon juices.

Another prosperous research method was applied to analysis of lime juice samples obtained from different locations of Iran. LOD, LOQ, linear range, recovery, repeatability of retention times, and peak areas for hesperidin, diosmin and eriocitrin were 0.0283-0.0512 µg/mL, 0.0857-0.155 µg/mL, 0.0283-105.0 µg/mL ($R^2 > 0.99$), 93.3 - 98.1, 3.2 - 4.7 and 2.8 - 3.6 %, respectively [29]. As can be seen, the current achieved data have high level in range of flavonoid as compared with Saeidi *et al* [29] but the eriocitrin has lower content range (0-24.02).

According to Iranian standards [30] the approved amounts of hesperidin, diosmin and eriocitrin in lemon juice are a minimum of 90, 13, and 20 µg/mL but there aren't any accurate levels of quercetin. Consequently, all studied groups are unacceptable for their hesperidin and eriocitrin levels with the exception of no. 1 (104.84 ± 12.58 µg/mL). Furthermore, natural group, nos.1 and 2 have logical amount of approved diosmin but based on eriocitrin point of view. All the studied groups showed less amount of Iranian standard [30].

CONCLUSION

This work focused on the analytical aspects of lemon juice compounds as well as on their implications for the food industry and the relevance of lemon for nutrition and health. The natural lemon group had greater amount of total antioxidant capacity and also higher flavonoid contents than the other group. It should be stressed that, although the reported limits of Iranian standard(hesperidin 90, diosmin 13, eriocitrin 20 µg/mL)must be reviewed by the regulatory agency, but developed method can be applied to determine fake lemon juices.

ACKNOWLEDGEMENT

This work was extracted from the student thesis of one of our dissertation which was supported by a grant (no. 91-04-33-11300) from the Research Council of Tehran University of Medical Sciences, Tehran, Iran.

REFERENCES

1. *Hajimahmoodi M, Aliabadipoor M, Moghaddam GH, Sadeghi N, Oveisi MR, Jannat B. Evaluation of in vitro antioxidant activities of lemon juice for safety assessment. America J Food Technol 2012; 7: 708-717.*
2. *Valentine IK, Maria VK, Bruno B. Phenolic cycle in plants and environment. J Cell Mol Biol 2003; 2: 13-18.*
3. *Tomas-Barberan F, Espin JC. Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables. J Sci Food Agr 2001; 81: 853-876.*
4. *Ghasemzadeh A, Jaafar HZE. Anticancer and antioxidant activities of Malaysian young ginger (Zingiber officinale Roscoe) varieties grown under different CO2 concentration. J Med Plant Res 2011; 5: 3247-3255.*
5. *Gao K, Henning SM, Niu Y, Youssefian AA, Seeram NP, Xu A, Heber D. The citrus flavonoid naringenin stimulates DNA repair in prostate cancer cells. J Nutr Biochem 2006; 17: 89-95.*
6. *Yoshimizu N, Otani Y, Saikawa Y, Kubota T, Yoshida M, Furukawa T, Kumai K, Kameyama K, Fujii M, Yano M, Sato T, Ito A, Kitajima M. Anti-tumour effects of nobiletin, a citrus flavonoid, on gastric cancer include: Antiproliferative effects, induction of apoptosis and cell cycle deregulation. Aliment Pharmacol Ther 2004; 20: 95–101.*
7. *Du QZ, Chen H. The methoxyflavones in Citrus reticulata Blanco cv. ponkan and their antiproliferative activity against cancer cells. Food Chem 2010; 119: 567–572.*
8. *Kukic J, Petrovic S, Niketic M. Antioxidant activity of four endemic Stachys taxa. Biolog Pharma Bulletin 29: 725-729.*
9. *Galeotti F, Barile E, Curir P, Dolci M, Lanzotti V. Flavonoids from carnation (Dianthus caryophyllus) and their antifungal. Phytochem Lett J 2008; 1: 44-50.*
10. *Spencer JPE, Flavonoids: Modulators of brain function? BJN 2008; 99 (E-S1): 60–77.*
11. *El-Shahawi MS, Bashammakh AS, El-Mogy T. Determination of trace levels of diosmin in a pharmaceutical preparation by adsorptive stripping voltammetry at a glassy carbon electrode. J Anal Sci 2006; 22: 1351-1354.*
12. *Hirata A, Murakami Y, Shoji M, Kadoma Y, Fujisawa S. Kinetics of radical-scavenging activity of hesperetin and hesperidin and their inhibitory activity on COX-2 expression. Anticancer Res 2005; 25: 3367-3374.*
13. *Wong CC, Li HB, Cheng KW, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem J 2006; 97: 705–711.*
14. *Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Antioxidant activities of peel, pulp, and seed fractions as determined by FRAP assay. Nutr Res J 2003; 23: 1719-1726.*

15. Rice-Evans CA, Bourdon R. Free radical lipid interaction and their pathological consequences. Prog Lipid Res 1993; 12: 71-110.
16. Jayaprakasha GK, Girennavar B, Patil BS. Radical scavenging activity of Rio red grapefruits and sour orange fruit extracts in different in-vitro model system. Bioresource Technol 2008; 4484-4494.
17. Prasad NK, Divakar S, Shivamurthy GR, Aradhya SM. Isolation of a free radical scavenging antioxidant from water spinach (Ipomoea aquatica Forsk). J Sci Food Agr 2005; 85: 1461-1468.
18. Amin I, Norazaidah Y, Hainida KIE . Antioxidant activity and phenolic content of raw and blanched Amaranthus species. Food Chem 2006; 94: 47-52.
19. Li H, Wang X, Li Y, Li P, Wang H. Polyphenolic compounds and antioxidant properties of selected China wines. Food Chem 2009; 112: 454-460.
20. Ayub Ali M, Inaotombi Devi L, Varij Nayan Kh, Chanu V, Ralte L. Antioxidant activity of fruits avalable in Aizawl market of Mizoram, India. Int J Pharm Biol sci 2010; 1: 76-81.
21. Davis W, Lamson MS, Matthew S, Brignall ND. Antioxidants and cancer III: quercetin. Altern Med Rev 2000; 5: 196-208.
22. Xu GH, Liu DH, Chen JC, Ye XQ, Ma YQ, Shi J. Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chem 2008; 106: 545–551.
23. Scambia G, Ranelletti FO, Panici PB. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast cancer cell line: P-glycoprotein as a possible target. Cancer Chemother. Pharmacol 1994; 34:459-464.
24. Wu T, Guan Y, Ye J. Determination of flavonoids and ascorbic acid in grapefruit peel and juice by capillary electrophoresis with electrochemical detection. Food Chem J 2007; 100: 1573–1579.
25. Petersona JJ, Beecherb GR, Bhagwatc SA, Dwyera JT, Gebhardtc SE, Haytowitzc, DB et al. Flavanones in grapefruit, lemons, and limes: A compilation and review of the data from the analytical literature. J Food Compos Anal 2006; 19: 74-80.
26. Galati EM, Monforte MT, Kirjavainen S, Forestieri AM, Trovato A, Il Farmaco. Biological effects of hesperidin, a citrus flavonoid. Antiinflammatory and analgesic activity. Farmaco1994; 49: 709-712.
27. Fadlinizal Abd Ghafar M, Nagendra Prasad K, Weng KK, Ismail A. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. AJB 2010; 9: 326-330.
28. Ramful D, Tarnus E, Aruoma OI, Bourdon E, Bahorun T. Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. Food Res Int 2011; 44: 2088-2099.
29. Saeidi I, Hadjmohammadi MR, Peyrovi M, Iranshahi M, Barfi B, Babaei AB, Dust AM. HPLC determination of hesperidin, diosmin and eriocitrin in Iranian lime juice using polyamide as an adsorbent for solid phase extraction. J Pharma Biomed Anal 2011; 419-422.
30. Iran-Standard, 2014 [Cited 2013 Mar 8]. Available from: http://www.isiri.org.