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Research Article

Standardization of *Tragopogon graminifolius* DC. Extract Based on Phenolic Compounds and Antioxidant Activity

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Tragopogon graminifolius DC. (TG), Compositae family, is traditionally used for the treatment of various diseases like gastrointestinal and hepatic disorders. The aim of the present study is to standardize extracts from TG used for preparation of different dosage forms in traditional Iranian medicine (TIM) based on phenolic compounds. For this purpose, total phenolic content and some phenolic compounds were determined in ethanolic extracts from aerial part and root of TG by HPLC method. Furthermore, antioxidant activity was evaluated using DPPH-HPLC methods. Caffeic acid, gallic acid, ρ-coumaric acid, ferulic acid, and catechin were detected in root and aerial part of TG. ρ-Coumaric acid ($6.357 \pm 0.014\ mg{\cdot}g^{-1}$) was dominant phenolic compound in aerial part followed by ferulic acid ($1.24 \pm 0.018\ mg{\cdot}g^{-1}$). Also, ρ-coumaric acid ($2.685 \pm 0.031\ mg{\cdot}g^{-1}$) was highly abundant in root, followed by catechin ($2.067 \pm 0.021\ mg{\cdot}g^{-1}$). Antioxidant activity of root extract ($460.45 \pm 0.78\ \mu g\ Vit.E{\cdot}mL^{-1}$) was better than that of aerial part. Generally, phenolic compounds are one of the major constituents of TG and could be used as markers for standardization of dosage forms prepared from this plant. Also, TG demonstrated significant antioxidant activity using DPPH-HPLC method. Phenolic compounds of TG may be responsible for its marked antioxidant properties.

1. Introduction

Tragopogon graminifolius DC., Compositae family, is known as "Sheng" or "Lahiat-o-tis" in traditional Iranian medicine (TIM). Its aerial part including leaves and stems is widely consumed as green vegetable and in various indigenous foods in the west of Iran. It also has been used for treatment of wounds, hemorrhage, and various gastrointestinal and hepatic disorders in TIM. Traditional medicine sources asserted that root part possesses higher biological activity than the other parts of the plant [1–3]. In folk medicine of other countries, different species of *Tragopogon* have been used as anticough, astringent, vulnerary, and skin repairing and proposed to be beneficial for gastrointestinal disorders [4, 5]. Acute toxicity on TG demonstrated that this plant has a wide range of safe consumption [3]. The main constituents of *Tragopogon* genus are phenolic compounds [6–8]. Triterpene saponins, bibenzyls, and dihydroisocoumarins have been also reported from *Tragopogon* genus [9, 10]. Since various dosage forms are prepared from the extracts of aerial part and root part of this plant in TIM, we aimed to standardize the extracts from aerial part and also root of TG based on some phenolic

compounds by HPLC method. Moreover, antioxidant activity and total phenol content of the extracts were evaluated using DPPH-HPLC and spectrophotometry methods, respectively.

2. Material and Method

2.1. Chemical and Reagents. Caffeic acid, gallic acid, ρ-coumaric acid, ferulic acid, catechin, DPPH, α-tocopherol, acetic acid, sodium bicarbonate, Folin-Ciocalteu reagent, ethanol, and methanol (HPLC gradient grade) were purchased from Merck (Darmstadt, Germany).

2.2. Plant Material. TG was collected in July 2012 from Kermanshah province, west of Iran, and authenticated by Dr. F. Attar (Department of Biology, Faculty of Sciences, University of Tehran), and a voucher specimen (number 43603) was deposited in the Central Herbarium of Tehran University. Plant aerial part (leaves and stems) and roots were separately dried in the shade at room temperature and ground to powder.

2.3. Plant Extraction. Powdered aerial part (100 g) and root (100 g) were separately extracted three times with 70% ethanol. Their extracts were filtered and evaporated at reduced pressure to yield residues about 29.45% and 18.86% on the basis of dried plant material, respectively. Samples in concentration of 1 mg/mL in HPLC grade deionized water were assessed.

2.4. Total Phenolic Content Assay. Total phenolics were determined using Folin-Ciocalteu reagent [11]. The extract (1 mL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min. 1.5 mL sodium bicarbonate solution (60 g/L) was added to the mixture and after incubation for 90 min at room temperature; the absorbance was measured at 750 nm using a UV-visible spectrophotometer (GBC, Cintra 40). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions (25–150 μg/mL in 80% methanol). The results were calculated as gallic acid equivalent (GAE) per one gram dry extract and reported as mean value ± SD.

2.5. Liquid Chromatographic Analysis. Samples were analyzed by a Knauer HPLC (Germany) system which consisted of a pump (Maxi-Star K-1000, Knauer, Germany), a degasser, an automated injector, a column oven, and a UV detector. The system was controlled by Euro Chrom 2000 software (Version 1.6, Knauer Co., Germany). Chromatographic conditions were evaluated and optimized in Eurospher-100 C8 column (5 μm, 4.6 × 250 mm). Column temperature was maintained at 60°C. Mobile phase consisting of 2% (v/v) acetic acid in water-methanol, 82 : 18 (v/v), and the flow rate was set at 1.5 mL/min. The injection volume for all samples was 10 μL and the chromatographic detection was monitored at 280 nm. Phenolic compounds were identified according to retention times as a comparison with the standards. The concentration of each phenolic compound was measured from peak area according to calibration curves. The amount of each phenolic acid was expressed as milligram per gram of dry extracts.

2.6. Antioxidant Capacity Measured by DPPH-HPLC Method. Antioxidant activity of ethanol extracts from TG aerial part and root was determined using DPPH free radical scavenging activity, according to Hajimahmoodi et al. [11]. Fifty microliter of each sample was added to 2 mL DPPH (0.1 mmol/L) solution. Mixtures were shaken and then remained in dark at room temperature for 40 min. The samples were filtered through a 0.2 μm Minisart RC 4 membrane filter (Sartorius, Germany); then 10 μL was injected to HPLC. Chromatographic conditions were evaluated in Eurospher-100 C8 column (5 μm, 4.6 × 250 mm) eluted isocratically by mixture of methanol: deionized water; 90 : 10 as a mobile phase at a flow rate of 1 mL·min^{-1}. The chromatograms were monitored at 517 nm. 50 μL of distilled water was added to 2 mL of DPPH as blank sample. The differences in the reduction of DPPH peak area between blank and samples were taken to determine the percent of radical scavenging activity of the samples.

2.7. Method Validation. Based on the ICH method [12], the detection limit (LOD) and quantitation limit (LOQ) were expressed as DL = $3.3\sigma/S$ and QL = $10\sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve of the analyte. The estimate of σ was carried out by using the standard deviation of blank. In this study, blank samples were analyzed three times, and the magnitude of the analytical background response was measured. Then the standard deviation of responses was calculated. In order to verify the feasibility of the method, sample recovery was used by analyzing samples before and after the addition of known quantities of each compound.

2.8. Statistical Analysis. Three replicates of each sample were used for statistical analysis and the values were reported as mean ± SD. The mean values were compared by t-test. Differences at P value < 0.05 were considered to be significant.

3. Results and Discussion

3.1. HPLC Method Validation. Some important method validation parameters are presented in Table 1. Results for correlation coefficients were always larger than 0.995 showing a good relationship between peak areas and concentrations. Detection limits of phenolic compounds in the sample were also found to be satisfactory. The accuracy of the method was validated by analyzing a spiked sample. The recoveries of these standards were between 92.24 and 107.21%.

3.2. Total Phenolic Content. Total phenolic content of aerial and root extracts of *T. graminifolius* were determined using Folin-Ciocalteu method. The results which were presented in Table 2 show that total phenolic content of the aerial extract

Table 1: Validation parameters of applied HPLC method.

Compounds	Precision (%)		Accuracy (%)	Slope	Correlation coefficient (%)	LOD (mg/mL)	LOQ (mg/mL)
	Intraday	Interday					
Gallic acid	1.01	2.17	94.83	5.623	0.9959	0.105	0.319
Catechin	2.59	3.09	106.02	0.988	0.9939	0.130	0.393
Caffeic acid	2.46	2.32	105.14	3.719	0.9950	0.135	0.408
ρ-Coumaric acid	0.72	2.87	92.24	8.770	0.9905	0.162	0.490
Ferulic acid	1.46	2.93	107.21	4.943	0.9957	0.109	0.329

Table 2: Phenolic compounds and antioxidant activity of *T. graminifolius* ethanolic extract in root and aerial part determined by HPLC.

Phenolic compound[1]	Root extract	Aerial extract[2]
Gallic acid	0.748 ± 0.016	0.873 ± 0.027
Catechin	2.067 ± 0.021*	1.079 ± 0.033*
Caffeic acid	0.135 ± 0.006*	0.326 ± 0.021*
ρ-Coumaric acid	2.685 ± 0.031*	6.357 ± 0.014*
Ferulic acid	1.363 ± 0.023*	1.24 ± 0.018*
Antioxidant activity[3]	460.45 ± 0.78*	242.61 ± 0.46*
Total phenol[4]	292.34 ± 13.67*	560.67 ± 18.85*

[1] mg·g extract dry weight^{-1}.
[2] Results are expressed as means of three replicates ± S.D.
[3] μg Vit.E.E·mL^{-1}.
[4] mg·g^{-1} as gallic acid equivalents.
* Values are significantly different (t-test, $P < 0.05$).

(560.67 ± 18.85 mg·g^{-1} as gallic acid equivalent) was significantly higher than the root extract (292.34 ± 13.67 mg·g^{-1} as gallic acid equivalent). Mroueh et al. showed that the total phenolic content of methanol extract from *T. porrifolius* aerial part was 37.1 (mg·g^{-1} as gallic acid equivalent) [13]. Therefore, both *T. graminifolius* aerial and root part possess higher phenolic content than *T. porrifolius* aerial part.

In comparison with other medicinal plants, Surveswaran et al. expressed that total phenolic content of various Indian plants ranged from 0.6 (*Acacia Arabica*) to 414.7 (*Acacia catechu*) mg·g^{-1} as gallic acid equivalents [14]. In another study in which total phenolic content of selected Chinese medicinal plant was measured, the range of total phenolic content was between 2.4 (*Gynostemma pentaphyllum*) and 457.0 (*Acacia catechu*) mg·g^{-1} as gallic acid equivalents [15]. So, *T. graminifolius* encompasses a high abundant level of total phenolic content by regarding the other assessed plants.

3.3. Determination of Phenolic Compounds. Phenolic compounds like flavonoids, phenolic acids, and polyphenols are the most important in *Tragopogon* genus constituents. The HPLC method was applied to evaluate four phenolic acids and one flavonoid including caffeic acid, gallic acid, ρ-coumaric acid, ferulic acid, and catechin in root and aerial part of *T. graminifolius*. Figure 1 shows the chromatogram of phenolic compound in aerial part and also in standards. The high amount phenolic compound in 70% ethanolic extract of aerial part was ρ-coumaric (6.357 ± 0.014 mg·g^{-1}) followed by ferulic acid (1.24 ± 0.018 mg·g^{-1}), while the most phenolic compound in root was ρ-coumaric acid (2.685 ± 0.031 mg·g^{-1}) followed by catechin (2.067 ± 0.021 mg·g^{-1}).

Statistical results from t-test showed that all the phenolic compounds are significantly different in root and aerial with exception of gallic acid amount (Table 2). The amount of catechin (2.067 ± 0.021 mg·g^{-1}) in root was significantly higher than aerial part (1.079 ± 0.033 mg·g^{-1}). Also, ferulic acid in root (1.363 ± 0.023 mg·g^{-1}) was significantly more than the aerial (1.24 ± 0.018 mg·g^{-1}). In contrast, aerial part possesses higher ρ-coumaric acid (6.357 ± 0.014 mg·g^{-1}) and caffeic acid (0.326 ± 0.021 mg·g^{-1}) than root part (2.685 ± 0.031 and 0.135 ± 0.006 mg·g^{-1}, resp.). Various phenolic compounds have been identified in *Tragopogon* genus plants: apigenin, luteolin, quercetin 3-O-β-D-glucoside, vitexin, isovitexin, vicenins-1 and 2, swertisin, orientin, isoorientin, and lucenins-1 and 2 have been detected in five species of *Tragopogon* including *T. porrifolius*, *T. mirus*, *T. dubius*, *T. miscellus*, and *T. pratensis* [6]. Also chlorogenic acid, 4,5-dicaffeoylquinic acid, scopoletin 7-oglucoside, vitexin, and orientin have been found in *T. porrifolius* aerial part [8]. In Smolarz and Krzaczek study [16], detected flavonoids in *T. orientalis* L. were apigenin, vitexin, luteolin, orientin, isoorientin, and quercetin. In another study performed by DAD UV-Vis HPLC, the amounts of ferulic acid (197.79 μg·g^{-1}), caffeic acid (278.72 μg·g^{-1}), resveratrol (13.95 μg·g^{-1}), sinapic acid (110.85 μg·g^{-1}), rutin (89.99 μg·g^{-1}), and gallic acid (1347.85 μg·g^{-1}) were determined in *T. pratensis* flower and gallic acid was the dominant, followed by caffeic acid [7]. Therefore, the content of ferulic acid and caffeic acid in *T. graminifolius* aerial part was more than *T. pratensis*. In contrast, gallic acid in *T. pratensis* was higher than that of *T. graminifolius* [7].

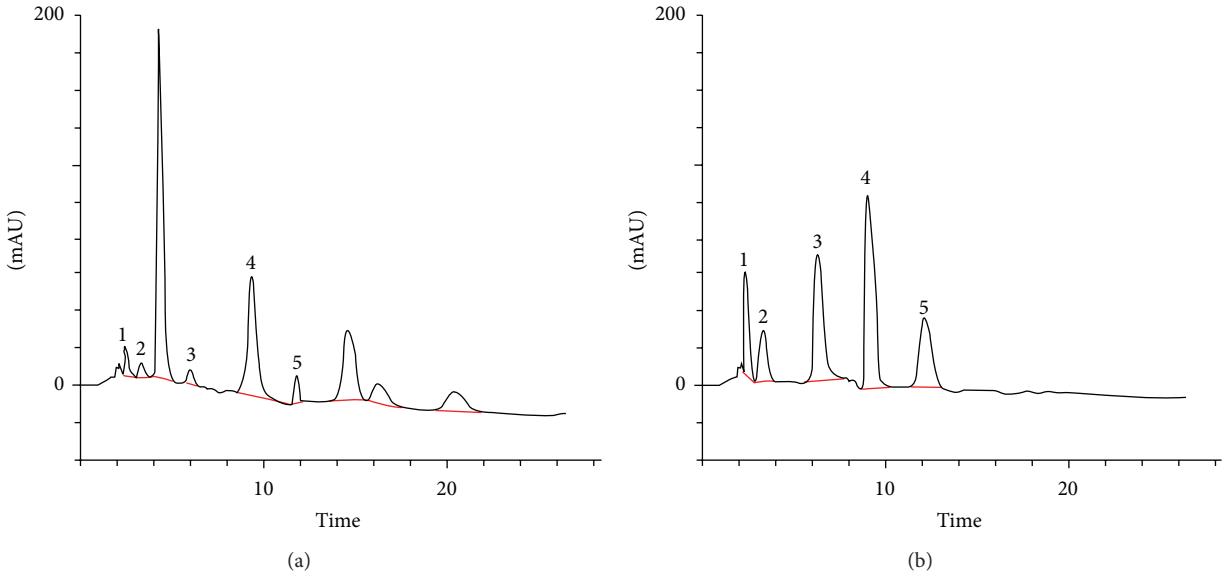

Figure 1: HPLC chromatogram of phenolic compounds from *T. graminifolius* 70% ethanolic extract of aerial part (a) and standard phenolic compounds mixture (b). Chromatogram identification: (1) gallic acid; (2) catechin; (3) caffeic acid; (4) ρ-coumaric acid; (5) ferulic acid.

Among other medicinal plants, the amount of caffeic acid in *T. graminifolius* aerial part is more than many herbs including *Petroselinum sativum* (0.144 mg·g^{-1}), *Glycyrrhiza glabra* (0.153 mg·g^{-1}), *Epilobium hirsutum* (0.231 mg·g^{-1}), *Salvia officinalis* (0.074 mg·g^{-1}), *Ginkgo biloba* (0.398 mg·g^{-1}), *Origanum majoricum* (0.104 mg·g^{-1}), *Poliomintha longiflora* (0.081 mg·g^{-1}), *Thymus vulgaris* (0.117 mg·g^{-1}), *Rosmarinus officinalis* (0.029 mg·g^{-1}), and *Polygonum aviculare* (0.215 mg·g^{-1}). In contrast, content of caffeic acid in *Taraxacum officinale* (0.726 mg·g^{-1}), *Tanacetum vulgare* (8.94 mg·g^{-1}), and *Humulus lupulus* (0.381 mg·g^{-1}) is higher than *T. graminifolius* [17, 18].

According to Wojdyło et al. study, ρ-coumaric acid ranges from 0.021 (*Taraxacum officinale*) mg·g^{-1} to 1.25 mg·g^{-1} (*Juglans regia*) in different selected herbs, so the *T. graminifolius* in the current report possesses high content of ρ-coumaric acid both in its root and aerial parts [17]. Additionally, the amount of ρ-coumaric acid in *T. graminifolius* was higher than that of black mulberry (*Morus nigra*), white mulberry (*Morus alba*), and red mulberry (*Morus rubra*) [19].

By regarding the ferulic acid, *T. graminifolius* aerial and root part possess higher ferulic acid content than *Artemisia vulgaris* (0.138 mg·g^{-1}), *Inula helenium* (0.245 mg·g^{-1}), *Silybum marianum* (0.207 mg·g^{-1}), *Petroselinum sativum* (0.186 mg·g^{-1}), and *Glycyrrhiza glabra* (0.197 mg·g^{-1}). Moreover, the level of ferulic in *Melisa officinalis* (1.11 mg·g^{-1}) is comparable to *T. graminifolius*. *Tanacetum vulgare* (4.71 mg·g^{-1}) has dramatically dominant amount of ferulic acid in comparison with *T. graminifolius* [17].

In a study on some selected medicinal plants performed by Proestos et al. [20], gallic acid was ranged from 0.005 (*Lavandula vera*) to 0.41 mg·g^{-1} (*Vinca rosea*). So, amounts of gallic acid in both *T. graminifolius* aerial and root are more than these herbs. On the other hand, level of gallic acid of different extract from *Dimocarpus longan* and *Mangifera indica* was between 0.2 and 8.38 mg·g^{-1}, which exhibited a high amount of gallic acid [21].

The results also showed that *T. graminifolius* is rich with respect to the catechin amount both in root and aerial in comparison with black mulberry (*Morus nigra*), white mulberry (*Morus alba*), and red mulberry (*Morus rubra*) which were 0.075, 0.037, 0.086 (mg·g^{-1}), respectively [19]. Also, in Proestos et al. study [20], range of catechin was from 0.006 in *Asperula odorata* to 0.26 in *Mentha pulegium* which indicates high level of catechin in *T. graminifolius*.

3.4. Antioxidant Activity of T. graminifolius Extract. Figure 2 shows a chromatogram of DPPH absorbance peak in blank and sample. The DPPH scavenging activities of plant extracts were expressed as μg vitamin E equivalent (μg Vit.E.E·mL^{-1}). As data showed in Table 2, the 70% ethanolic extract of TG root (460.45 ± 0.78 μg Vit.E.E·mL^{-1}) exhibited higher antioxidant activity than that of aerial part (242.61 ± 0.46 μg Vit.E.E·mL^{-1}). According to Mroueh et al. methanol extract from *T. porrifolius* aerial part showed significant antioxidant activity (744 μmol·g^{-1} as Fe^{2+} equivalent) through FRAP spectrophotometry method [13]. The antioxidant capacity (DPPH) of various Indian plants, studied by Surveswaran et al. [14], ranged from 2.2 (*Ricinus communis*) to 6796.9 (*Terminalia chebula*) (μmol·g^{-1} as Trolox equivalent). The DPPH scavenging activities of lemon juices (*Citrus lemon* L.) were expressed from 208.91 to 888.59 (μg Vit.E.E·mL^{-1}) in Hajimahmoodi et al. study [22]. Plant antioxidant activity, measured by the DPPH method in report of Wojdyło et al. [17], ranged from 0.073 in *Archangelica officinalis* to 20.21 (μmol·g^{-1} as Trolox equivalent) in *Epilobium hirsutum*. Therefore, *T. graminifolius* demonstrated a significant antioxidant activity which encompasses a key

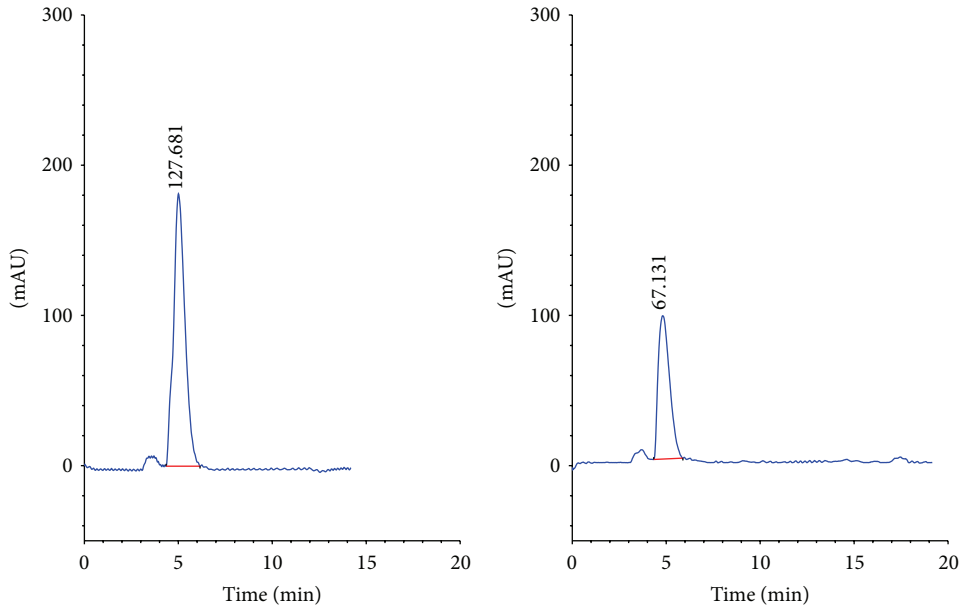

Figure 2: DPPH chromatogram of (a) blank and (b) sample.

role on various medicinal activity of this plant including protective and healing function on peptic and duodenal ulcer and also wound healing and skin repairing activity [2, 3].

Phenolic compounds in various investigations demonstrated a wide range of biological activities, the most important of which are antioxidant properties [11, 23, 24]. The higher antioxidant activity of root part in comparison with aerial part could be related to the role of higher abundant phenolic compounds in the root, including catechin and ferulic acid.

Generally, ethanolic extract of *T. graminifolius* root and aerial part possesses high level of total phenolic content and encompasses a wide range of phenolic components: caffeic, gallic, ρ-coumaric, ferulic acid, and catechin. Moreover, its potential antioxidant and radical scavenging function indicate the efficacious consumption of this remedy in different diseases.

4. Conclusion

This study focused on the standardization of TG based on phenolic compounds as well as its antioxidant activity. This study suggested a HPLC method for standardization of herbal drugs containing TG based on phenolic compounds. Also significant antioxidant activity was demonstrated by TG using DPPH-HPLC method. Phenolic compounds of TG may be responsible for its marked antioxidant properties.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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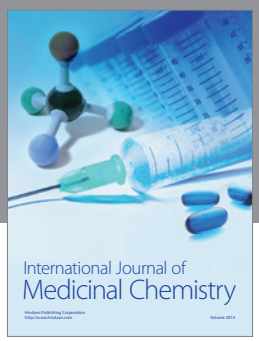

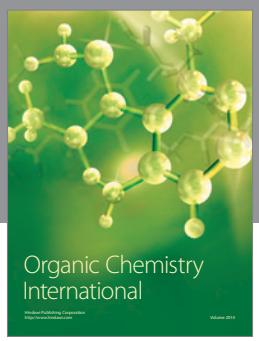

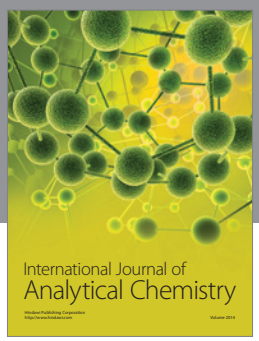

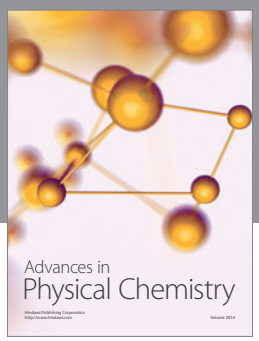

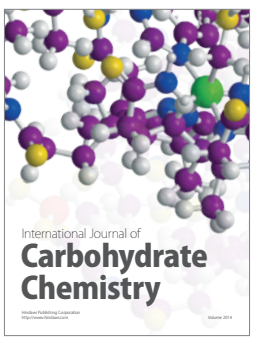

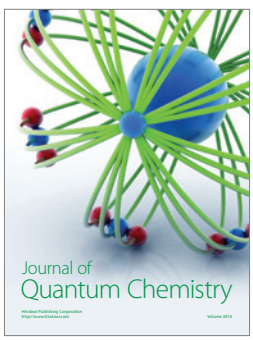

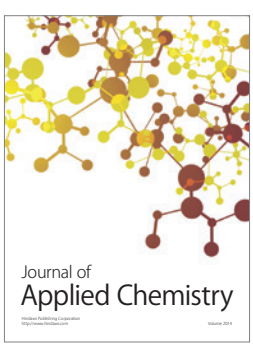

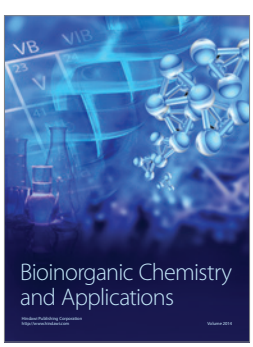

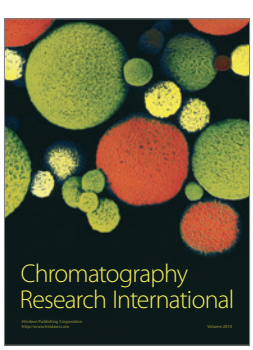

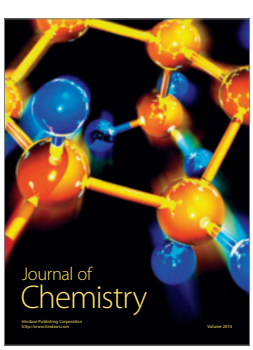

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