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Evaluation of antioxidant activities of *Mentha piperita* essential oils obtained by different extraction methods

M. Gavahian [1], R. Farhoosh [2]*, A. Farahnaky [3], Katayoun Javidnia[4], F. Shahidi[5]

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Abstract

As traditional extraction methods like Hydrodistillation (HD) and steamdistillation (SD) have long extraction times, some novel extraction methods like microwave-assisted hydrodistillation (MAHD) and ohmic-assisted hydrodistillation (OAHD) are recently introduced. In this study, essential oils of *Mentha piperita* were extracted by OAHD and MAHD and the results were compared with those of the SD and HD to clarify if these novel procedures have significant effect on antioxidant activities of extracted essential oils. The results showed that OAHD and MAHD are able to reduce extraction time (up to 72%) and also required electrical energy. Furthermore, all extracted essential oils were shown to have approximately same physical properties (relative density and visual color) and antioxidant activity using DPPH and β-carotene bleaching methods. The findings of this study revealed the applicability of using mint essential oil obtained by MAHD and OAHD as a natural antioxidant in food and pharmaceutical products.

Keywords: Essential oils, Hydrodistillaion, *Mentha piperita*, Microwave-assisted hydrodistillation, Ohmic-assisted hydrodistillation, Steamdistillation.

Introduction

The genus *Mentha* (family Lamiaceae), including more than 25 species, grows widely throughout the temperate regions of the world (Gulluce *et al*, 2007). *Mentha piperita* commonly known as peppermint frequently cultivated in many countries of East Asia, Europe, America and Australia for the production of essential oils (Gulluce *et al*., 2007 and Pandey *et al*., 2003). The essential oils and extracts from peppermint have been in use since ancient times for the treatment of many digestive tract diseases and in cuisines (Iscan *et al*., 2002).

Essential oils of mint aerial parts contain a large number of aromatic chemicals like menthol, menthone, isomenthone and menthofuran. These compounds are commercially important for the pharmaceutical, food, cosmetic and beverages industries (Carmines, 2002; Bakkali *et al*., 2008).

The peppermint oil is reported to have anti-oxidant properties, antibacterial activity and is one of the most important constituents of some over-the-counter remedies in Europe for irritable bowel syndrome (Pittler and Ernst, 1998; Singh *et al*., 2011).

As the living tissues are under the threat of damage by reactive oxygen derivatives which resulted from aerobic metabolism, it is therefore important to prevent oxidation. Such free radicals are usually short-lived species but they possess a single unpaired electron, rendering them highly reactive against biologically important macromolecules including DNA, proteins and membrane lipids. To counteract this threat to their integrity, cells have evolved a variety of defense systems based on both water-soluble and lipid-soluble antioxidant species, and on antioxidant enzymes. A high proportion of the antioxidant systems of the human body are dependent on dietary constituents.

The most widely used synthetic

1, 2 and 5. Former Ph.D Student, Associate Professor And Propessors Department of food and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Respectively.
3. Associate Propessor Department of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.
4. Propessor Medicinal & Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran.
(*- Corresponding Author Email: rfarhoosh@um.ac.ir)

antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA, propyl galate PG and tertiary butyl hydroquinone TBHQ) have been suspected to cause or promote negative health effects including mutagenic and carcinogenic consequence (Branen, 1975; Barlow, 1990; Namiki, 1990). Consequently, there is a growing interest in studies of natural additives as potential antioxidants. The search for natural antioxidants, especially of plant origin, has notably increased in recent years (Ebrahimzadeh *et al*., 2010).

There are a number of conventional methods for extracting essential oils, e.g. hydrodistillation (HD), steam distillation (SD) and organic solvent extraction (Presti *et al*., 2005). However, SD and HD methods suffer from some disadvantages including losses of volatile compounds and long extraction times and are known to be energy intensive methods. Furthermore, elevated temperatures can cause partial or full degradation of natural constituents especially monoterpenes which are vulnerable to structural changes under steam distillation conditions (Presti *et al*., 2005). Conventional solvent extraction method has introduced to involve losses of more volatile compounds during removal of the solvent (Moyler, 1991; Presti *et al*., 2005).

Because of disadvantages of traditional methods of extraction, researchers introduce and evaluate some alternative extraction methods such as microwave-assisted hydrodistillation (MAHD) and ohmic-assisted hydrodistillation (OAHD). MAHD was compared with HD in the extraction of essential oils from *Thymus vulgaris* L. (Golmakani and Rezaei, 2008). Gavahian *et al*. (2011) used a combination of ohmic heating and distillation for separation of essentials oils from *Zataria multiflora* Boiss (Shirazi Thyme) and found significant reductions in extraction time and consumed energy for ohmic-assisted hydrodistillation (OAHD) compare to conventional hydrodistillation method. This research team also reported similar result on OAHD for *Thymus vulgaris* and *Myrtus communis* (Gavahian *et al*., 2012; Gavahian *et al*., 2013).

Despite many studies reported on novel methods of extraction and also mint essential oils, there is no report which can clarify if different methods of extraction have significant effect on antioxidant activities of extracted essential oils from this medicinal herb. Therefore, the aim of this work was to use different techniques (including HD, SD, MAHD and OAHD) for the extraction of essential oils from dried peppermint areal parts and to compare the antioxidant activity of extracted essential oils.

Materials and methods

Plant materials

Fresh aerial parts of peppermint before flowering stage were collected from an indigenous crop in Noor-Abad (Mamasani, Sothern Iran), in July 2013. The herbs were then dried in a dark room under ambient conditions (30-40 °C) for four days on a large screened tray, packed in high density poly ethylene (HDPE) bags, put in a cardboard box and kept in a dark and cool place for further experiments. The moisture content of the plants was measured in triplicate using a laboratory oven by drying until reaching constant weight and was about 12.4±0.2%.

Steam distillation

SD was performed using a laboratory heater (MAG-K; Gerhardt Ltd., Germany; and 500 W) as the heating source (Presti *et al*., 2005). In SD procedures, 15 g of dried peppermint aerial parts placed in the junction of flask and Clevenger device. The bottom of the junction was perforated to let steam easily travel. 0.5 L distilled water were heated in the apparatus flask for up to 2 h from initial temperature of 27±1°C (similar to initial temperature of material in other studied extraction methods). The extraction process continued until no more essential oils were obtained and also required time to collect all extractable oil was recorded as total needed extraction time (i.e. the period of time between start of process and the time afterward no more essential oil collected). To remove water, the extracted essential oils were

then dried over anhydrous sodium sulfate and stored in amber vials at 4 °C for further experiments.

Hydrodistillation

HD is an approved method that is used as a reference for the quantification of essential oils (Stahl-Biskup, 2002). HD was carried out in a similar way as dicribed by Gavahian *et al*., 2012. Briefly, Fifteen grams of dried peppermint aerial parts with 0.5 L distilled water were put into HD with a Clevenger-type apparatus and essential oils were extracted until the time that no more essential oils were obtained. Removal of water from essential oils was performed as described for SD samples. The extracted essential oils were then stored in a cool (4 °C) and dry place for further experiments.

Ohmic assisted hydrodistillation

OAHD was performed using modified version of an ohmic distillator device with platinum electrodes as designed and developed by Farahnaky *et al*., 2010, in the Department of Food Science and Technology of Shiraz University. OAHD was performed at 220 V, 50 Hz and similar to that described by Gavahian *et al*., 2012.

In OAHD procedures, 30 g of dried peppermint aerial parts and 0.5 L salted water (1 NaCl%, w/v) were heated in the apparatus flask for up to 2 h from initial temperature of 27±1°C (similar to initial temperature of material in HD method). The extraction process continued until no more essential oils were obtained. Anhydrous sodium sulfate was used to remove water from the extracted essential oils and the dried essential oils were stored in amber vials at 4 °C for further experiments.

Microwave assisted hydro distillation

A domestic microwave oven (NN-S674MF, Panasonic, Japan, 32 l, 1100 W; variable in 110 W increments, 2.45 GHz) was modified for MAHD operation and the extractor performed similar to that described by Golmakani and Rezaei, 2008. Thirty grams of mint samples were placed in a 1 L flask containing salted water (500 ml, 1% v/v). The flask was setup within the microwave oven cavity and a Clevenger was used on the top (outside the oven) to collect the extracted essential oils. In addition, the volume and dimensions of the utilized container and condenser were exactly similar to that used for other studied extraction processes. The microwave oven was operated at 500 W power level for a period which was was sufficient to extract all the essential oils from the sample.

Removing of water from MAHD samples were conducted similar to the method described for OAHD samples. These dried essential oils were stored in amber vials at 4°C until they were used for analysis.

Energy Consumption during extraction

In all extraction methods, the amounts of energy consumption during extraction were monitored using the designed software. In addition, the input power consumption was monitored using a separate Wattmeter at the entrance of electrical heater power supply (i.e. at the entrance of ohmic apparatus power supply, microwave apparatus power supply and laboratory heaters of HD and SD power supplies). Afterward, the amount of required energy (kW) for extraction of one milliliter of mint essential oil in each extraction method (OAHD, MAHD, HD and SD) was calculated. These calculations were conducted similar to that was reported by Gavahian *et al*., 2011. Moreover, the equivalent amount of emitted carbon dioxide (as a green house gas) of consumed energy was calculated according to the literature: to obtain 1 kWh from coal or fuel, i.e. 800 g of CO_2 will be emitted to the atmosphere during combustion of fossil fuels (Ferhat *et al*., 2006).

Physical properties

Specific gravity of the essential oils from the mint samples were measured according to Food Chemical Codex (FCC) (FCC, 1996) at 25 °C. In addition, the color of the oils was determined visually as directed in FCC (FCC, 1996).

Antioxidant activity

Free radical scavenging activity

The antioxidant activity of the extracted peppermint essential oils were assessed by their ability to scavenging 2,2-diphenyl-1-picrylhydrazyl stable radicals (DPPH) by using the method described by Burits and Bucar, 2000. Briefly, 50 microlitres of different concentrations of the essential oils samples in methanol (10–60 mg/mL) were added to 5 mL of a 0.004% methanol solution of DPPH. After incubating for 30 min at room temperature and under dark condition, the absorbance of the samples was read against a blank at 517 nm using a spectrophotometer (Reyleigh UV9200, China). Inhibition of free radical DPPH in percent (I%) was calculated using Equation 1:

$$I\% = (A_{blank}-A_{sample}/A_{blank})\times 100 \quad (1)$$

Where A_{blank} is the absorbance of the blank (containing all reagents except the test compound) and A_{sample} is the absorbance of the test essential oil or BHT. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity, which was calculated from the plot of inhibition percentage against concentration. All tests and analysis were run in triplicate and averaged.

β-carotene-linoleic acid test

Antioxidant activities of extracted essential oils were also determined using β-Carotene bleaching assay. In this test, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. A stock solution of β -carotene-linoleic acid mixture was prepared as follows: 0.2 mg of β -carotene was dissolved in 10 mL of chloroform and 1 mL was added to 20 mg linoleic acid and 200 mg of Tween 40. Chloroform was gently removed under a stream of nitrogen gas. Then, 50 mL of distilled water, saturated with oxygen (30 minutes, 100 mL min^{-1}), was added with vigorous shaking. 200 µl of ethanolic stock solution of sample and BHT were separately mixed with 5 mL emulsion. Readings of all samples were taken immediately by spectrophotometer at t= 0 minute at 470 nm. The cuvettes were incubated in a water bath at 50ºC for 30 minutes. Then, absorbances of samples at 470 nm were determined by spectrophotometer (Zhang *et al*., 2006). All determinations were performed in triplicate and the results were averaged. The percentage inhibition was calculated using the equation 2:

$$\%Inhibition = (A_{sample(t)}-A_{control(t)})/(A_{control(0)}-A_{control(t)}) \times 100 \quad (2)$$

Where $A_{sample(t)}$ and $A_{control(t)}$ are the absorbance of the sample and control at t, respectively, and $A_{control(0)}$ is absorbance of the control at t= 0 minute.

Statistical analysis

All extraction processes were performed in triplicates. An independent samples t-test was performed to determine significant differences between the means using SPSS (version 19.0.0; IBM Institute Inc., USA).

Results and discussions

Comparison of extraction duration and yield

The required time for extraction essential oils from peppermint using different methods are presented in Table 1. As the data shows, both traditional methods (HD and SD) require approximately same time to perform the extraction process but more time than OAHD and MAHD methods. Before extraction time of 20 min, the novel studied methods (OAHD and MAHD) resulted in oil recovery to that obtained by HD after more than 55 minutes. In the other word, OAHD and MAHD require less than 32% time of that needed in SD and HD. Therefore, saving time is an obvious advantage of these new methods of extraction in comparison to traditional methods. This is due to higher extraction rate in OAHD and MAHD. In OAHD heating is applied through ohmic heating, which causes heat generation within the materials. Due to internal heat generation, the heating is faster than traditional

systems used for heating foods where heat must travel gradually from the outside surface of container (i.e. the surface of heater) to the inside material (Singh and Heldman, 2009). In contrast to conventional heating systems, microwave heating (which is employed in MAHD) penetrate materials, and heating extends within the entire food material. The rate of heating is therefore more rapid (Singh and Heldman, 2009). Although the mechanism of heating in OAHD and MAHD is completely different, in this study both of them resulted in similar extraction duration.

There are a number of parameters that can influence the essential oil content of aromatic herbs including harvest time, ecological and climatic conditions (i.e. Clark *et al*., 1980; Baranauskiene, 2003; Tabatabaie and Nazari, 2007). It was previously reported that the oil content of peppermint can vary by harvest time from 0.72% to more than 3% and oil yield of peppermint depends on growing stage (White *et al*., 1987). In this study the resulted yields in all methods were in the range of the previous reports on peppermint. As presented in Table 1, SD yields lower amount of essential oils than other studied methods. The lower yield in SD process can be related to packing of mint leaves in junction of steam distillatory device (i.e. the part of device where mint areal parts were placed) which can prevent leaves from perfect extraction.

Comparison of energy consumption

In terms of time and energy, the reduced cost of extraction is clearly an advantage for the studied novel extraction methods (OAHD and MAHD) (Tables 1 and 2). The traditional methods (HD and SD) require long extraction times (about one hour) while OAHD and MAHD need approximately third amount of this time. The energy requirement to perform the extraction, based on the power consumptions of the electromantle for 1 ml of extracted oils, was 0.7 kWh and 0.8 kWh for HD and SD while this value was 0.1 kWh for OAHD and 0.2 kWh for MAHD, respectively. This indicates a substantial saving in the extraction cost by novel studied extraction methods (especially OAHD) compared to the conventional extraction techniques (HD and SD). Regarding environmental impacts, the calculated quantity of carbon dioxide (the primary greenhouse gas) emitted to the atmosphere is higher in the case of HD (551 g CO_2/ml of essential oils) and SD (668 g CO_2/ml of essential oils) than for OAHD (108 g CO_2/ml of essential oils) and MAHD (170 g CO_2/ml of essential oils). In other words, the emitted CO_2 for extraction of equal volume of essential oils by studied novel extraction methods are far less than that of emitted by studied tradition extraction methods.

Based on the findings of this research and the previously reported data on OAHD and MAHD, OAHD and MAHD are therefore suggested as "environmentally friendly" extraction methods (from energy consumption view point). However, unlike MAHD, OAHD lacks the risk of radiation leakage and its hazards to the operators.

Physical properties evaluation

The physical properties (specific gravity and color) of mint essential oils extracted by SD, HD, OAHD and MAHD are shown in Table 1. There is no significant difference between both traditional and novel studied methods for the specific gravity. Every essential oil has a typical range of densities at specified temperatures. Generally, the densities of essential oils range from 0.780 to 0.970 $g.cm^{-3}$ (Bowles, 2003). Similarly, sensory color perceptions of all samples were similar and within the range indicated by Food Chemical Codex (FCC).

From the physical tests of the extracted essential oils, it can be concluded that OAHD and MAHD, as possible substitutions techniques for traditional methods, did not introduce any considerable changes to the studied physical properties of the extracted essential oils from mint aerial parts.

Antioxidant activity

The extracted essential oils of peppermint by studied methods were explored for antioxidant activity by evaluating their IC_{50}

(from DPPH assay) and Inhibition of linoleic acid peroxidation (from β-carotene bleaching assay), and the results are given in Table 3.

Table 1. Effect of extraction methods on total needed extraction time, yield and physical properties of mint essential oils.

	SD	HD	OAHD	MAHD
Total extraction time (min)	$58.75^{a} \pm 2.85$	$55.88^{a} \pm 3.30$	$18.54^{b} \pm 2.23$	$16.50^{b} \pm 1.01$
Yield (%V/W)	$2.00^{b} \pm 0.27$	$2.29^{a} \pm 0.16$	$2.25^{a} \pm 0.10$	$2.17^{a} \pm 0.14$
Relative density	$0.91^{a} \pm 0.01$	$0.91^{a} \pm 0.01$	$0.91^{a} \pm 0.01$	$0.91^{a} \pm 0.01$
Visual color	Pale yellow	Pale yellow	Pale yellow	Pale yellow

* The same letters in each row indicate that the means are not significantly different (p <0.05).

Table 2. Effect of extraction method on energy consumption.

	SD	HD	OAHD	MAHD
Total electrical energy consumption (kWh)	0.50^{a}±0.02	0.47^{a}±0.03	0.09^{c}±0.01	0.14^{b}±0.01
Electric consumption (kWh/ml essential oils)	0.835^{a}±0.095	0.689^{a}±0.081	0.135^{c}±0.011	0.212^{b}±0.020
emitted CO_2 (g/ ml essential oils)	668.3^{a}±76.1	551.2^{a}±64.8	108.3^{c}±8.7	169.8^{b}±16.4

* Values are Mean ± SD (n-=3) of each *Mentha peppireta*, analyzed individually in triplicate. The same letters in each row indicate that the means are not significantly different (p <0.05).

Table 3. Effect of extraction methods on the antioxidant activity of *Mentha piperita* essential oils

	SD	HD	OAHD	MAHD	BHT
DPPH, IC_{50}, (µg mL^{-1})	10.4^{a}±0.6	9.8^{a}±0.9	9.9^{a}±0.9	9.6^{a}±0.7	5.2^{b}±0.4
Inhibition of linoleic acid peroxidation (%)	40.34^{b}±2.9	40.8^{b}±3.1	41.0^{b}±3.0	41.0^{b}±3.7	91.8^{a}±1.2

* Values are Mean ± SD (n-=3) of each *Mentha peppireta*, analyzed individually in triplicate. The same letters in each row indicate that the means are not significantly different (p <0.05).

Totally, lower IC_{50} value reflects a better protective action. IC_{50} values of the extracted essential oils (using SD, NHD, OAHD and MAHD) have been compared with BHT. The free radical-scavenging activities of all extracted essential oils were approximately similar (9.6±0.7 µg.mL^{-1} for MAHD *vs.* 9.8±0.9, 9.9±0.9 µg.mL^{-1} and 10.4±0.6 for HD, OAHD and SD, respectively). In addition, antioxidant activity of BHT as positive control was compared in a parallel experiment that showed lower IC_{50} value (i.e. higher antioxidant activity) with IC_{50} of 5.2±0.4 µg.mL^{-1}.The similarity in antioxidant activity of extracted essential oils by studied methods revealed that new proposed extraction methods which have been studied in this study did not considerably affect the IC_{50} value of extracted essential oils. The IC_{50} of mint essential oils are approximately two times greater than IC_{50} of BHT. Similar results have been reported before. For example Singh *et al*., 2011 reported an IC_{50} for *M. pepperita* essential oil to be twice of that of BHT (15.2±0.9 vs 6.1 ±0.3, respectively). The most powerful scavenging compounds were reported to be monoterpene ketones (menthone and isomenthone) and 1,8-cineole (Mimica-Dukic *et al*., 2003).

The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (Tepe *et al*., 2005; Kulisic *et al*., 2004). Therefore, antioxidant activities of the extracted essential oils in comparison with synthetic antioxidants (BHT) were evaluated (Table 2). As can be seen in Table 2, there are no significant differences between the antioxidant activities of all extracted essential

oils (41.0±3.0 for OAHD *vs.* 40.34±2.9, 40.8±3.1 and 41.0±3.7 for SD, HD and MAHD, respectively). Moreover, antioxidant activity of BHT was approximately two times greater than *M. piperita* oil. This theme has also been reported by other researchers. For instance, Yadegarinia *et al*. (2006) reported that the percent of inhibition in β -carotene bleaching test of mint oil was 50.17 while this value was 86.75 for BHT. The results were almost consistent with the data obtained from the DPPH test. Generally the essential oils rich in phenolic compounds exhibited significant antioxidant activity (Lu and Foo, 2000).

These properties are in high demand by the food industry in order to find possible alternatives to synthetic chemicals (namely BHT, phenolics). Oxidative stress is involved in the pathogenesis of numerous diseases. These results suggest that the mint essential oils have a potent antioxidant activity. In addition using OAHD and MAHD as alternative methods for extraction of mint essential oil will not significantly vary its antioxidant properties.

Conclusion

In this study, novel extraction methods (OAHD and MAHD) resulted in a reduced extraction time and extraction energy compared to the conventional techniques. After 16 min of MAHD extraction, it was possible to collect almost all the existing essential oils of the mint (2.2%, indeed), whereas in SD process, after 59 min, less amount of essential oils was extractable (2.0%, indeed). Essential oils obtained by all studied novel and traditional methods were almost similar in their antioxidant activities. MAHD and OAHD can be considered as applicable alternative methods of extraction since they require significantly less amounts of time and energy to operate. In comparison to traditional SD, not only could they increase oil yield but also resulted in the oils with similar antioxidant activities.

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ارزیابی فعالیت آنتی‌اکسیدانی روغن‌های اساسی نعناع فلفلی (*Menthapiperita*) با روش‌های متفاوت استخراج

محسن گواهیان[۱]- رضا فرهوش*[۲]- عسگر فرحناکی[۳]- کتایون جاویدنیا[۴]- فخری شهیدی[۵]

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چکیده

به دلیل طولانی بودن زمان استخراج روش‌های سنتی از جمله تقطیر با آب (HD) و تقطیر با بخار آب (SD)، اخیراً روش‌های جدیدی مانند تقطیر با آب در حضور امواج مایکرو (MAHD) و تقطیر مقاومتی (OAHD) معرفی شده‌اند. در پژوهش حاضر، روغنهای اسانسی نعناع فلفلی (*Menthapiperita*) به روش‌های OAHD و MAHD استخراج و فعالیت آنتی‌اکسیدانی آنها با روغن‌های اسانسی حاصل از روش‌های SD و HD مقایسه شد. نتایج حاکی از آن بود که روش‌های OAHD و MAHD سبب کاهش زمان استخراج (تا ۷۲ درصد) و نیز انرژی الکتریکی لازم می‌شوند. علاوه بر این، تمام روغن‌های اسانسی استخراج شده دارای خواص فیزیکی (دانسیته نسبی و رنگ) و فعالیت آنتی‌اکسیدانی (روشهای DPPH و رنگبری بتا-کاروتن) تقریباً یکسانی بودند. یافته‌های این تحقیق نشان داد روغن اسانسی نعناع فلفلی حاصل از روش‌های OAHD و MAHD را می‌توان به عنوان فراورده‌ای آنتی‌اکسیدانی در مواد غذایی و دارویی مورد استفاده قرار داد.

واژه‌های کلیدی: روغن‌های اسانسی، تقطیر با آب، نعناع فلفلی، امواج مایکرو، تقطیر مقاومتی، تقطیر با بخار آب.

۱، ۲ و ۵: به ترتیب دانش آموخته دکتری و استادان گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه فردوسی مشهد.
۳: دانشیار. گروه علوم و صنایع غذایی. دانشکده کشاورزی. دانشگاه شیراز. شیراز.
۴: استاد. مرکز تحقیقات شیمی دارویی و گیاهی دانشگاه علوم پزشکی شیراز. شیراز.
(*- نویسنده مسئول : Email: rfarhoosh@um.ac.ir)