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Sequential Microwave-assisted Extraction for Isolation of Quercetin from Red Kidney Bean

S. Aghajanian[a], S. kazemi[b], S. Esmaeili[a], S. Aghajanian[c], A. A. Moghadamnia*[b]

[a] *School of pharmacy, Islamic Azad University of Amol, Amol, Iran*
[b] *Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
[c] *School of Engineering Science, LUT University, Lappeeranta Finland*

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A B S T R A C T

Quercetin is a biological flavonoid, which can be found in red kidney bean at high concentration. In this study, a comparison was conducted between traditional and modern extraction methods. Sequential microwave-assisted extraction was used for isolation of quercetin from red kidney bean. The effect of several factors such as particle size of grinded kidney bean, extraction solvent, microwave power and extraction time on the extraction yield of quercetin was considered. To analyze the quercetin, HPLC method was developed. The highest extraction efficiency was obtained (35.8 mg quercetin /g kidney bean) when 60 w/w% acetone used as the solvent. In addition, the solvent to solid ratio was 10:1 and the irradiation power of 800W was applied for the experiment duration of 1 min. Acquired extraction yield according to the current method was higher than extracted quercetin from soxhlet (24.6 mg quercetin /g kidney bean) and maceration (32.75 mg quercetin /g kidney bean). In the present study, quercetin was successfully extracted from kidney bean by microwave-assisted extraction. Quercetin structure remains intact and can be used to produce therapeutic compounds.

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1. INTRODUCTION

Phaseolus vulgaris L, also known as the common bean is an herbaceous annual flowering plant. Different types of common beans that are cultivated for edible purposes are navy beans, kidney beans, red beans, black beans, pinto beans and can berry beans [1]. They belong to the Fabaceae or leguminaceae family that is widely distributed in all over the world.

Among all of the major food beans, the common bean is the world's third most important bean after soybeans (Glycine max (L.) Merr) and peanut (Arachis hypogea L.) [2]. Common bean is cost effective source for proteins, fibers, carbohydrates, minerals, vitamins, iron and it has a significant role in human nutrition.

There are several reports concerning bean intake and reduced risk of cardiovascular disease, diabetes mellitus, obesity, cancer and diseases of the gastrointestinal tract [1, 3, 4]. These potential health aspects of beans have been recognized for existence of secondary metabolites like polyphenolic compounds, which maintain antioxidant properties [5-8].

According to literatures [2, 9], kidney bean contains flavonoids as key polyphenolic compound. Quercetin ($C_{15}H_{10}O_7$) with the chemical structure shown in Figure 1 is a biological flavonoid, which can be found in onion, black tea, broccoli, carrot [10] and kidney bean [11]. Quantitative HPLC analysis of quercetin among 20 different bean samples was conducted in India, demonstrate that the dark red small kidney beans have a higher concentration of quercetin [12]. Several beneficial properties have been reported for quercetin such as neuroprotective and anticancer effects, antiviral, anti-inflammatory effects and inhibits platelet aggregation [13-16].

Since producing high quality pharmaceutical ingredients is still a challenging task, these industries are always in search of economical and viable products and

*Corresponding Author Email: *aliamoghadamnia@gmail.com* (A. A. Moghadamnia)

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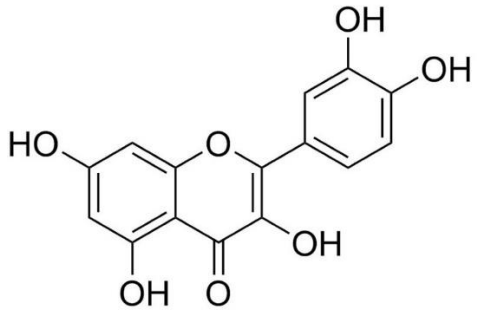

Figure 1. chemical structure of quercetin

materials to use in the manufacturing processes. Utilizing plant sources as a raw material could be one of the solutions to these demands.

As reported by Azmir et al [17], according to nature of the plant, solvent, temperature, pressure as well as time of the extraction methods can be different from one another. They divided the techniques of extraction into conventional and unconventional methods. Soxhlet extraction, maceration and distilling are considered as the conventional techniques of extraction. Extraction by utilizing ultrasonic waves, microwave devices, supercritical fluids, accelerated solvents are considered as unconventional and modern methods.

Use of domestic microwave oven for laboratory work was first started by Samra in 1975 for analysis biological samples [18]. Since then, various experiments have been conducted by utilizing microwave radiation as a method of extraction of organic compounds [19, 20]. Non-ionized electromagnetic microwaves, which are located in the mid-range of infrared and x-rays electromagnetic spectrum, have frequencies between 300 MHz to 300 GHz. [19, 21].

Various studies [20, 22-26] have been carried out for comparing efficiency of microwave assisted extraction (MAE) with traditional techniques, such as solvent maceration and Soxhlet. They observed that large amounts of solvent and longer times are required for traditional methods, while MAE techniques result in more material in less time. Consequently, microwave assisted extraction has been introduced as a useful technique for plant material extraction due to the purity of the extracted material in short extraction time and lower energy consumption.

Karacabey [27] conducted an experiment on extraction of Thymoquinone by means of the MAE method. Factors such as temperature, extraction time and the ratio of solvent to solid were considered as the most significant variables that affects the process. Selection of a suitable solvent that has the capability to dissolve the bioactive compound and absorb the energy of microwave device is another essential factor that needs to be considered in MAE processes [28].

In this study, MAE was used to extract quercetin from red kidney bean and factors that affect the extraction yield were examined. Through variation in temperature and radiation energy of the device, the most appropriate solvent and operating conditions for quercetin extraction were obtained. Eventually, to prove the advantages of this novel method, extracted quercetin from MAE, Maceration and Soxhlet methods were compared by the HPLC.

2. MATERIALS AND METHODS

High quality red kidney bean (Phaseolus vulgaris L.) was purchased from local market in Amol, Mazandaran, Iran. It was soaked in water over night, then it turned into smaller particles by Moulinex mill. Ethanol, acetone, HPLC grade acetonitrile as well as standard quercetin (> 98%), which used for UV and HPLC analyses, were provided by Merck (Darmstadt Germany). A domestic LG microwave oven with a maximum output power of 800 W was used for this study.

2. 1. Microwave-assisted Extraction of Quercetin In order to extract quercetin by microwave, 10 grams of grinded red kidney beans mixed with a constant solvent to solid ratio of 10:1. Throughout the extraction process, different purities of ethanol and acetone as well as water were used as solvents. Several tests were carried out at variable microwave output powers within the range of 160 to 800 W. Beaker containing the solution was kept in microwave for different durations from 30 seconds to 2 minutes. During the experiments, beaker was rotated to ensure an equal distribution of heat in samples. Since extensive heating causes the solvent to pour outside the beaker and for avoiding any solvent waste, heating was intermittent [20, 29]. At the end of each run and before conducting the HPLC tests, samples were taken out of the microwave and filtered. Drying process is done after filtration inside a laboratory oven at a temperature of 45C in a 24-hour time span.

2. 2. Soxhlet Extraction of Quercetin Soxhlet extraction as one of the traditional methods of extraction, was done to compare the efficiency of microwave-assisted extraction with traditional methods. 10 g of grinned red kidney beans were placed in a thin cellulose sheet and then transferred to a Soxhlet system. Based on the results of HPLC and spectrophotometry, acetone 60 w/w% was selected as the optimum solvent. 250 ml of 60w/w%acetone poured in the apparatus. The system is fixed at 70 ° C and after the flow of water, extraction is performed for 6 hours. At end of the experiments and after conducting the downstream processes (filtration, drying, etc.), samples were analyzed by HPLC to quantify the amount of quercetin.

2. 3. Maceration Extraction of Quercetin Maceration is one of the primary methods of extraction that is performed to compare quercetin extracted from Soxhlet and Microwave methods. In this method, 10 g of grinned red kidney beans with 250 ml of acetone 60 w/w% as an extracted solvent, are combined in the Erlenmeyer and then placed in a shaker for 72 hours. After this time, the sample is removed and transferred to the oven. After 24 hours, the dried sample was analyzed by HPLC for quercetin quantification.

2. 4. Analytical Techniques UV-VIS spectrophotometer (Jenway - 6300) analysis was carried out to determine the existence of quercetin within the extracted MAE samples. Additionally, high-performance liquid chromatography (HPLC, Smartline, Knauer, Germany) equipped with Eurospher II 100-5 C18 column and UV detector 2500 series was used for the analysis of quercetin. The mobile phase was composed of acetonitrile and water (80:20) which was used at the flow rate of 1 ml/min at 15 °C. Quercetin was detected at the wavelength of 260 nm; that was used to identify and quantify the amount of quercetin under investigation. For this purpose, initially, a calibration procedure for the HPLC was carried out by injecting standard solution of quercetin to obtain the appropriate calibration plot. Then, the unknown sample with a specific concentration (100 ppm) is injected to the HPLC to identify and acquire a peak, which corresponds to the amount of quercetin within the sample mixture in a given time.

3. RESULTS AND DISCUSSION

3. 1. Effects of Solvent Extraction In general, plants contain various types of chemical compounds that are biologically active in themselves and also in other organisms. This property of plants, especially the medicinal plants, have made them an alternative and/or a supportive medicine to use worldwide. Currently, the global market value of medicinal plant products exceeds $100 billion per annum [30]. Thus, ensuring the quality, safety and purity of a medicinal plants and herbal drugs have become a major subject in various industries.

Extraction method plays a significant role in the quality of final active ingredients of a plant. Within that process, identifying the type of solvent to use is a crucial step. Solvent should have the capability to effectively dissolve the particle in itself.

Quercetin, as a hydrophobic compound can be dissolved in lipophilic solvents. On the other hand, water due to its polarity is not an appropriate solvent for dissolution of quercetin. In the current work, 60 and 70 w/w% of ethanol, acetone as well as water are used as solvents. Figure 2 shows the effect of solvent on the extraction yield of quercetin while radiation time and power are kept constant. Acetone at 60 W/W% and ethanol at 70 w/w% resulted in the highest yield, respectively.

3. 2. Effect of Microwave Power and Irradiation Time Effect of irradiation power of the microwave on the extraction process is investigated and plotted in Figure 3. For this purpose, acetone 60 w/w%, which gives the highest extraction yield was selected as the primary solvent during the 1-minute process. Multiple microwave radiation power in the range of 160 to 800 W was applied to sample. Based on the acquired results of the experiments, maximum yield wa achieved at the highest radiation rate. Higher radiation power facilitates the destruction of the particle's cell wall, which in turn increases the solvent penetration rate within the cell and results in a higher extraction yield.

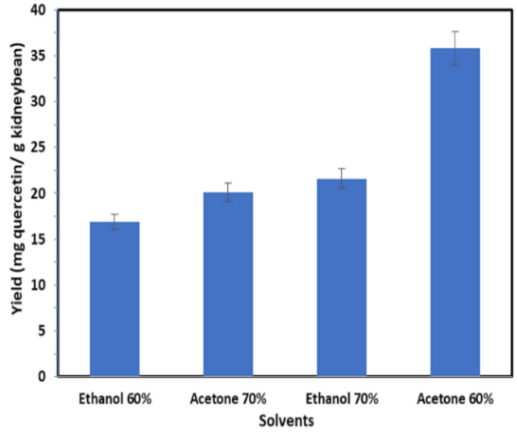

Figure 2. Effect of solvent on the extraction yield (10g of grinned bean particles, solvent to solid ratio 10:1)

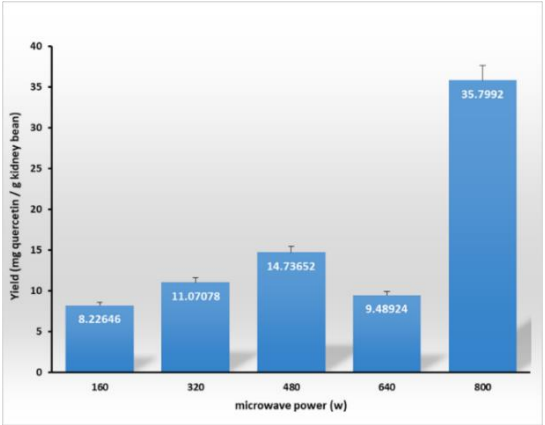

Figure 3. Effect of radiation power on the extraction yield (microwave time 1 min) The extraction condition was: 10g of grinned bean particles, 60% w/w acetone as solvent, solvent to solid ratio 10:1

As stated earlier, time of the whole process in the microwave-assisted extraction remarkably improves. Time of the process was changed from 30 seconds to 2 minutes while radiation power was kept constant at 800 W and acetone 60 w/w% was employed as the main solvent. Results are displayed in Figure 4. Based on the observations, efficiency of the extraction is increased from 25.2mg at 30 secondsto its maximum value of 35.8 mg quercetin /g kidney bean at 1-minute. After reaching the maximum value at 1-minute, any increase in extraction time reduces the yield of the product.

3. 3. Characterization of the Extracted Quercetin

HPLC is used for measuring the amount of quercetin extracted from red kidney bean. Figure 5 displays the chromatography results of 100 ppm quercetin after conducting the HPLC analysis. The inset shows the same concentration of standard quercetin. Based on the results, purity of extracted quercetin sample from the MAE is 75.3 %.

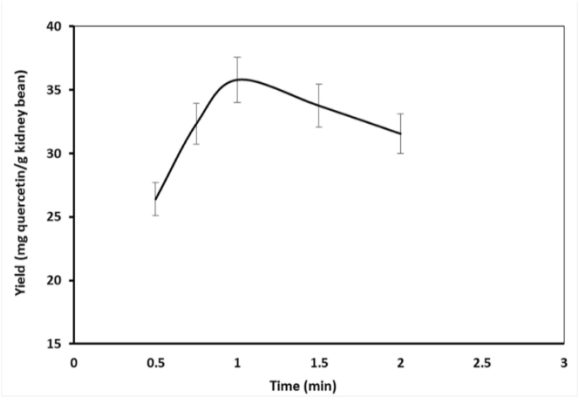

Figure 4. Effect of radiation time on the extraction yield (microwave power 800w) The extraction condition was: 10g of grinned bean particles, 60% w/w acetone as solvent, solvent to solid ratio 10:1

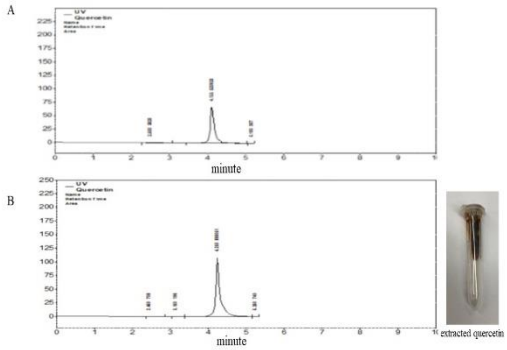

Figure 5. HPLC chromatogram of extracted (A) and standard (B) quercetin; the inset shows the chromatogram of the standard quercetin

The FTIR spectra of and extracted the standard quercetin are illustrated in Figure 6. In the spectrum of the standard quercetin, the band centered at 2936 cm−1 correspond to aromatic C–H stretching vibrations. The peaks at 996, 1250 and 1650 cm−1 are generally attributed to the CH2 wagging vibrations, asymmetrical stretching of=C–O–C and aromatic stretching of C=C (benzene ring), respectively. The FTIR spectrum of the extracted quercetin showed similar characteristic peaks, signifying the high purity of the extracted quercetin. The peak observed at 3550 cm−1 is confidently assigned to the O–H stretching bond.

3. 4. Comparison of Sequential Microwave-Assisted Extraction with Other Extraction Methods

Experiments with soxhlet and maceration techniques, as traditional methods of extraction, were also carried out along with the current MAE approach in order to make a broader comparison of their respective efficiency. As summarized in Table 1, yields of the three extraction methods are evaluated as follows: 35.8, 32.75 and 24.6 mg quercetin/g kidney bean for MAE, maceration and soxhlet, respectively. According to the acquired results, additional amount of quercetin is extracted in a shorter time (1 minute) in comparison to its conventional counterparts, which require hours of time and a higher energy consumption. Moreover, high

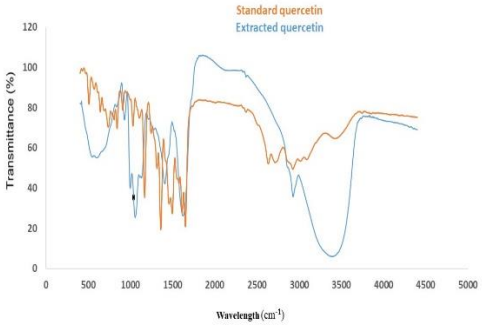

Figure 6. FTIR spectra of the standard and extracted quercetin

TABLE 1. Comparison of the extraction yields of 60%w/w acetone from different extraction methods

Extraction technique	Yield (mg quercetin/ g kidney bean)	Conditions
Soxhlet	24.6	Extraction time: 6 h
Maceration	32.75	Extraction time: 72 h
Sequential microwave	35.8	Extraction time: 1 min; microwave power: 800 W

amount of solvent was also used in traditional methods which is an undesirable factor. Eventually, it is shown that the efficiency of MAE method is approximately 32% higher than the common traditional methods, such as the soxhlet.

4. CONCLUSIONS

In this paper, sequential microwave-assisted extraction method is employed for extraction of quercetin from red kidney bean. Experiments are carried out at several operating conditions, and effects of variation of essential parameters on extraction yield was investigated. In order to optimize the amount of extraction, four major factors are analyzed, and their values are quantified as follows: type of solvent (60 w/w% acetone), solvent to solid ratio (10:1), microwave time (1-min), and microwave power (800W). Under this optimal condition, the acquired extraction yield is 35.8 mg quercetin/g kidney bean and the purity of the extracted quercetin is 75.3%. By comparison to the Soxhlet method, yield of the current extraction process is 32% higher. As shown in this work, this approach due to its lower energy consumption, short amount of process time and significant reduction in solvent waste is considered as an efficient and robust method of extraction when compared to its traditional counterparts. Additionally, this set-up could pave the way toward more economical and environment-friendly isolation of the bioactive compounds from medicinal plants.

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[a] *School of pharmacy, Islamic Azad University of Amol, Amol, Iran*
[b] *Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
[c] *School of Engineering Science, LUT University, Lappeeranta Finland*

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چکیده

کوئرستین یک فلاونوئید بیولوژیکی است که می‌توان آن را در لوبیای قرمز با غلظت زیاد یافت. در این مطالعه، ما مقایسه‌ای بین روش‌های سنتی و مدرن استخراج انجام دادیم. از روش استخراج متوالی به کمک مایکروویو برای جداسازی کوئرستین از لوبیای قرمز استفاده شد. تأثیر عوامل مختلفی از قبیل اندازه ذرات لوبیا خرد شده، حلال استخراج، توان مایکروویو و زمان بر عملکرد استخراج کوئرستین در نظر گرفته شد. از روش HPLC برای آنالیز کوئرستین استفاده شد. دراین روش از یک ستون C18 و فاز متحرک استونیتریل و آب استفاده میشود که طول موج، ۲٦۰nm و سرعت جریان، ۱ میلی لیتر در دقیقه می باشد. زمانی که استون٪ ٦۰ به عنوان حلال استفاده شد، بالاترین بازده استخراج (۳٥.۸ میلی گرم کوئرستین / گرم لوبیا قرمز) بدست آمد. علاوه بر این ، نسبت حلال به جامد ۱۰: ۱ و توان تابش w۸۰۰ در طول آزمایش ۱ دقیقه اعمال شده است. عملکرد استخراج در این روش بالاتر از بازده سوکسله (۲٤/٦ میلی گرم کوئرستین / گرم لوبیا قرمز) و ماسراسیون (۳۲/۷٥ میلی گرم کوئرستین / گرم لوبیا قرمز) بود. در مطالعه حاضر ، کوئرستین با استفاده از مایکروویو به راحتی از لوبیا قرمز استخراج شد. همچنین ساختار کوئرستین دست نخورده باقی ماند و می‌توان از آن برای تولید ترکیبات درمانی استفاده کرد.

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