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

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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
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 overnight, 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 45°C 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 grinded 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 the 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 grinded 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 was 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 grinded bean particles, solvent to solid ratio 10:1)](image-url)

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

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=O–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 4](image-url) Effect of radiation time on the extraction yield (microwave power 800w) The extraction condition was: 10g of grinnned bean particles, 60% w/w acetone as solvent, solvent to solid ratio 10:1

![Figure 5](image-url) HPLC chromatogram of extracted (A) and standard (B) quercetin; the inset shows the chromatogram of the standard quercetin

![Figure 6](image-url) 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.

5. ACKNOWLEDGEMENTS

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**Abstract**

Quercetin is a polyphenolic phytochemicals extracted from red kidney beans (Phaseolus vulgaris) and its quantification in red kidney beans is performed by high-performance liquid chromatography (HPLC). In the current study, the extraction of quercetin from red kidney beans was performed with microwave assisted extraction (MAE) and conventional extraction (CE). The effects of different parameters such as particle size, solvent concentration, microwave power and time were assessed. MAE was performed in a sequential mode.

**Keywords**

Kidney Bean, Flavonoid, Microwave Extraction, Purification

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