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Research Article

Comparison of Different Methods in Quercetin Extraction from Leaves of *Raphanus sativus* L.

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ABSTRACT

Background: *Raphanus sativus* (Radish) is a plant of the Brassicaceae family which is grown worldwide. This plant has been used in Iranian traditional medicine as a laxative, abortifacient and also recently as anti-tumor, anti-proliferative and anti-diabetic agent.
Quercetin, the most important flavonoid which is found in this plant, serves as an antioxidant and as a result an anticancer agent.
Methods: The present study was designed to compare extraction efficiency of different methods with various solvents in quercetin extraction from *R. sativus* leaves. The analysis of extracts was performed using high performance liquid chromatography (HPLC) and the quantification was carried out by the use of quercetin calibration curve.
Results: In consequence, Ultrasonic Assisted Extraction (UAE) method at 50% of ultrasound intensity (Frequency of 50/60 KHz) for 10 min in methanol proved to be the most efficient technique in quercetin extracting (11.8% yield).
Conclusions: This study demonstrated the optimized condition for quercetin extraction from *R. sativus* leaves, which is due to promoting larger agitation (cavitation) in UAE method.

Introduction

Raphanus sativus L. also known as Radish, is grown all over the world. However, the origin of *R. sativus* is in Southeast Asia. The plant possesses fruits which are 10–250 mm long, with lyrate or rosette shaped leaves and racemes pink or white flowers.[1]

Different parts of *R. sativus* have been investigated and reported to contain secondary metabolites including terpenoides, coumarins, flavonoids, anthocyanins, isothiocyanates,[2] saponins, alkaloids,[3] ascorbic acid, folic acid and also potassium[4] which indicates its medicinal benefits. In addition, low concentrations of saturated fat and cholesterol have been found in the leave's extract of the mentioned plant.[4]

R. sativus is rich in flavonoids such as quercetin, kaempferol, myricetin and apigenin.[5]

The plant leaves were reported to have spasmogenic effect in guinea-pig ileum and colon.[6] Moreover, cholinergic activity of the plant leaves extract has been reported.[4]

Furthermore, *R. sativus* possess antibacterial,[7] antioxidant,[8] anti-tumor,[9] anti-mutagenic,[10] anti-proliferative[11,12] and anti-diabetic[13] activities.

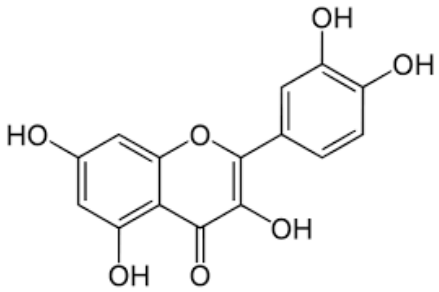

Figure 1. Quercetin chemical structure.

Presence of polyphenolics such as quercetin, etc. in the leaves and stem of *R. sativus* has been reported.[14]

Quercetin (Figure 1) is a plant-derived flavonoid, which is generated by plant metabolism and it is widely found in medicinal plants.[15] It has been also demonstrated that quercetin has many beneficial effects on human health, including anticancer, antioxidant, neuroprotective, antitumor[16] anti-viral[17] and anti-inflammatory[18] activities.

Quality of extracts and contents of active ingredients are influenced by factors such as

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extraction procedure, solvent used for extraction and solvent ratio. Therefore, to obtain high efficiency for medicinal plant extraction, it would be necessary to optimize extraction procedures.

Beside conventional methods, there are some novel techniques that are used to extract medicinal plants. Maceration, percolation, thermal digestion and soxhlet are some of the conventional methods, while modern methods include Microwave Assisted Extraction (MAE), Ultrasonication Assisted Extraction (UAE), Super critical Fluid Extraction (SFE), Solid Phase Micro Extraction (SPME) and etc. Most of the conventional methods are more time and solvent consuming in comparison with modern techniques.

Due to the presence of large amounts of quercetin in red radish leaves,[19] in this study different procedures were applied to extract a particular compound, quercetin, from *R. sativus* leaves, followed by extraction method optimization. Extracts were directly subjected to HPLC in order to compare efficacy of quercetin extraction.

Maceration involves placing the plant in a closed container with a suitable solvent and allowed to remain at room temperature for several days with frequent shaking. Time consuming from hours up to several days and using large volume of solvent, are the main disadvantages of this extraction method.[20]

Simple maceration may associate with gentle heat (35-40 ºC), to increase the extraction efficiency which is called digestion.

In UAE method, solid particles are vibrated, biological membranes are collapsed and extractable compounds are released into the solvent under ultrasonic waves.[21] Shorter reaction time, decreasing the release of toxic organic solvent to the environment and simply to carry out the operation, are the main advantage of UAE method.[22,23] The disadvantage of this method is that in some cases, active ingredients could be decomposed by ultrasound waves.[24]

In soxhlet method, the finely powdered plant is placed in a cellulose pocket in an extractor which is placed between a flask and a reflux condenser. The extracting solvent is added to the flask and heated in order to reflux and continuously extract the extractable compounds. Using large amounts of solvent and thermal decomposition of the target compound are the main disadvantages of this method.[24]

In this study we wish to report the optimized condition in terms of the method, solvent and temperature for quercetin extraction from *R. sativus* leaves.

Materials and Methods

Chemicals

All solvents used for HPLC analysis were HPLC grade. Ethanol, methanol, acetonitrile, chloroform and acetic acid were purchased from Merck Co. (Germany). Quercetin was purchased from Sigma-Aldrich Co. (England) and ultrapure water was applied to prepare all of the aqueous solutions.

Apparatus

Extract drying procedure was performed on a rotary evaporator (Heidolph, R-200, Germany) and a freeze dryer (Mitsubishi, Vaco, 5-11, Germany) at -80 ºC. An ultrasonic homogeneisator (Hielscher UP400S) was employed for plant extraction.

HPLC analysis was performed using a KNAUER liquid chromatograph, with an ODS Eurospher column (250 × 4.6 mm, 100–5; C18), which was protected by a C18 precolumn (Perfectsil Target, ODS-3 (5 µm)) and a 20 µl injection loop. A smartline Photodiode Array (PDA) detector 2850 (Knauer, Germany) was applied to detect analytes, and a chromgate software version 3.3, was applied for data processing.

Plant material

Aerial parts of *R. sativus* were purchased from a local herbal store located in Tehran, Iran (May 2015). Leaves were then separated and dried at room temperature for extraction purposes.

The mentioned plant was identified by Prof. G. Amin. Voucher specimen (PMP-412) of the plant was deposited in the Herbarium of Tehran University of Medical Sciences.

Sample preparation

Maceration

2 g of dried red radish leaves were placed in a flask and mixed with 40 ml solvent, including ethanol, methanol, water and chloroform separately. After placing the mixture for 1 day at room temperature with occasionally shaking, it was filtered, solvent evaporated at reduced pressure and the extract stored at 4℃.

Digestion

2 g of dried *R. sativus* leaves were mixed with 40 ml solvent including ethanol, methanol, water and chloroform separately and heated at 35-40 ºC for 1, 6 and 24 hr. The extract was then filtered, concentrated under vacuum and stored at 4 ºC.

Soxhlet Extraction (SE)

2 g of powdered leaves of *R. sativus* were placed individually in a soxhlet apparatus and were extracted with 80 ml of solvent with different polarities (ethanol, methanol, water and chloroform) for 24 hr. The extracts were then concentrated under reduced pressure using a rotary evaporator and stored at 4 ºC.

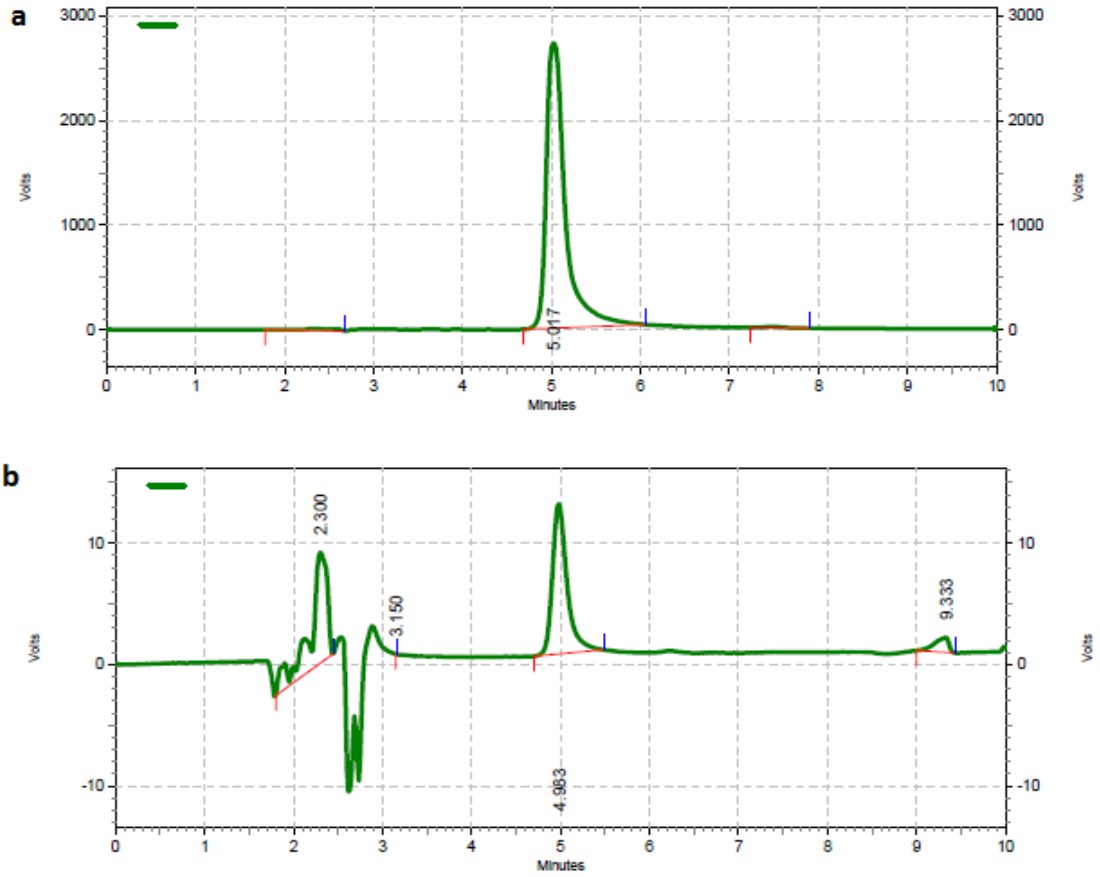

Figure 2. Chromatograms for a) quercetin standard solution and b) quercetin obtained by UAE method at 50% of ultrasound intensity.

Ultrasound Assisted Extraction (UAE)

An ultrasonic apparatus was used to extract 2 g of *R. sativus* leaves with 40 ml of the same above solvents for 1, 5 and 10 min at room temperature at 20%, 50% and 100% of ultrasound intensity (Frequency of 50/60 KHz). Regarding the optimization purposes, quercetin extraction with the best solvent (methanol), was then performed in 5, 10 and 15 min with different ultrasonic apparatus intensities. The extracts were then concentrated and stored at 4 ºC.

HPLC Condition

Previously reported method was used to determine quercetin in prepared extract with mobile phase composed of water–acetonitrile–acetic acid (50:44.9:0.1), at flow rate of 1 ml/min and the wavelength of 260 nm.[25] The chromatograms for quercetin standard solution and quercetin obtained by UAE method at 50% of ultrasound intensity are shown in Figure 2.

Validation of the method

The HPLC method was validated in terms of linearity and precision.

Linearity

The calibration curve was prepared using 5 concentrations of quercetin in the range of 1–30 µg/ml. There was a linear relationship between different concentrations of quercetin and area under curves (AUC) with formula of, y = 0.3464x – 0.0319; R^2 = 0.9994 (Figure 3).

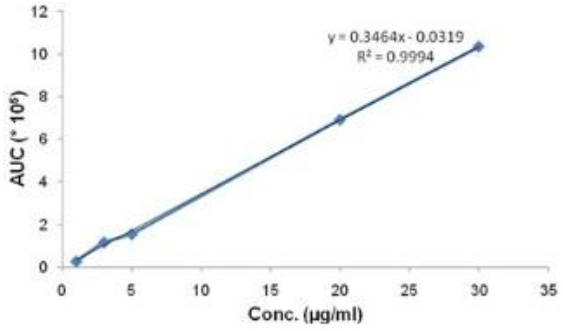

Figure 3. Linearity range of quercetin (1–30 µg/ml).

Precision

The precision of the method was figured out by inter-day and intra-day experiments. Inter-day experiments were performed on 5 different days by repeating analysis of five aliquots of the exact sample (CV% = 1.2) and also intra-day experiments in one day in the same way (CV% = 1.4).

Quercetin yield percentage

The quercetin extraction yield is a measure of the solvent's performance to extract quercetin from the original plant. It was defined as the concentration of quercetin recovered, compared with the extract concentration which has been subjected to HPLC.

Statistical analysis

All the analyses were carried out using the statistical software, Minitab v.14. A general factorial design with two factors (extraction methods with the best time and types of solvents) was performed.

As demonstrated in Figure 4a, UAE method with the solvent methanol provided the highest yield for quercetin extraction from *R. sativus* leaves regarding the comparison of mean values of 4 extraction methods with those of 4 solvents (Table 1). Also deviation from the parallelism in Figure 4a is the evidence of interaction between two factors.

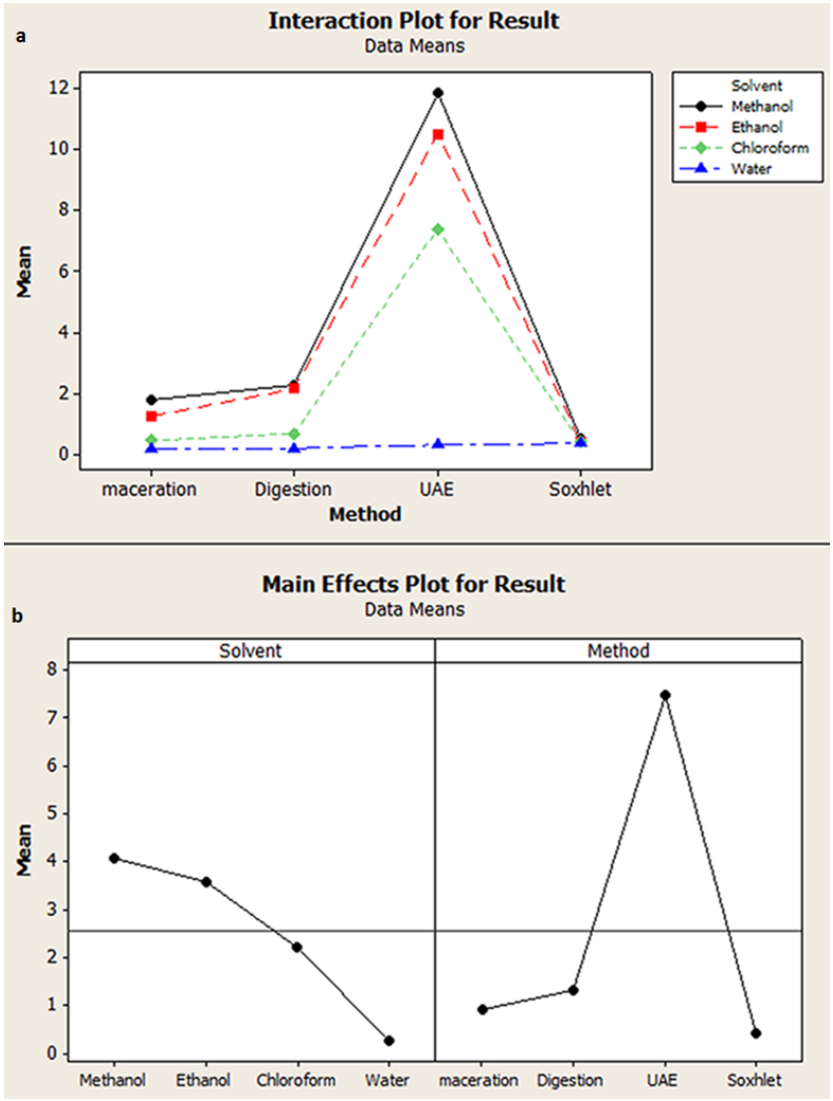

Figure 4. a) Interaction plot for result, b) Main effects plot for result.

Table 1. Analysis of Variance.

Source	Degrees of freedom	Sum of square	Mean of square	F-value	P-value
Solvent	3	104.605	34.868	5129.45	0.000
Method	3	396.745	132.248	19454.87	0.000
Solvent*Method	9	147.026	16.336	2403.19	0.000
Error	32	0.218	0.007		
Total	47	648.593			

S = 0.0824482 R-Sq = 99.97% R-Sq(adj) = 99.95%

Results and Discussion

The extraction yields of quercetin from *R. sativus* leaves have been determined using different methods including maceration, thermal digestion, soxhlet and UAE.

In addition to the method, the efficiency of extraction depends on solubility of the analytes in the solvent. Regarding the quercetin polarity, ethanol, methanol, water and chloroform were used for quercetin extraction.

Figure 5 shows the comparison of the quercetin extraction yields in different methods using different solvents. Our experiments indicated that methanol was the most efficient solvent system for all the above mentioned methods.

In 24 h extraction, digestion produced slightly higher yield (2.2%) than maceration (1.8%), which indicated that heating the mixture in digestion could increase extraction yields (Figure 5). However, only slight differences between the yields of the two above mentioned methods were observed. These results represented that along with increasing the yield in digestion method, decomposition of quercetin has been occurred.

Despite being time and large quantities of solvent consuming, soxhlet extraction has been used for 24 h. Yield of soxhlet method strongly depends on size of particles present in the sample. Small size particles allow solvent to diffuse into, and therefore, extraction time decreases and extraction yield increases. Soxhlet extraction method benefits an advantage of not being required filtration. There are some disadvantages such as long extraction times, using a large amount of solvent and therefore, long procedure for evaporation and concentration of the used solvent. Moreover, because of the long extraction time at the solvent boiling point, decomposition of the active compounds could be occurred. In present study, soxhlet extraction of *R. sativus* employing ethanol, methanol, water and chloroform as the solvent, resulted in very low yields, which indicated that decomposition of target has been occurred during long extraction time in solvent boiling point.

In plant cell walls, phenolic compounds form strong bonds with lignin, which consolidate them against digestion. The cross-linked network of phenolic compounds are tough to break down, and then methods such as UAE, which can destroy plant cell walls and facilitate releasing cell secondary metabolites, are more efficient than other techniques in extracting flavonoids such as quercetin. Therefore, sonication time and irradiation power should be considered carefully because excessive sonication can ruin the active components in extract.

The advantages of UAE are reduction in extraction time and in solvent consumption and also being inexpensive and easy in operation. In this study, UAE technique along with methanol as the extraction solvent in 10 min, produced the highest yield in quercetin extraction and also considerable yield differences while using solvents in different polarities.

The best extraction technique (UAE) was optimized in terms of time and ultrasound intensity. Several tests using various irradiation power (20%, 50%, 100% intensity) and time of irradiation (5, 10, 15 min) were made to optimize the procedure of UAE. The best result was obtained at 10 min and 50% of ultrasound intensity (Figure 6).

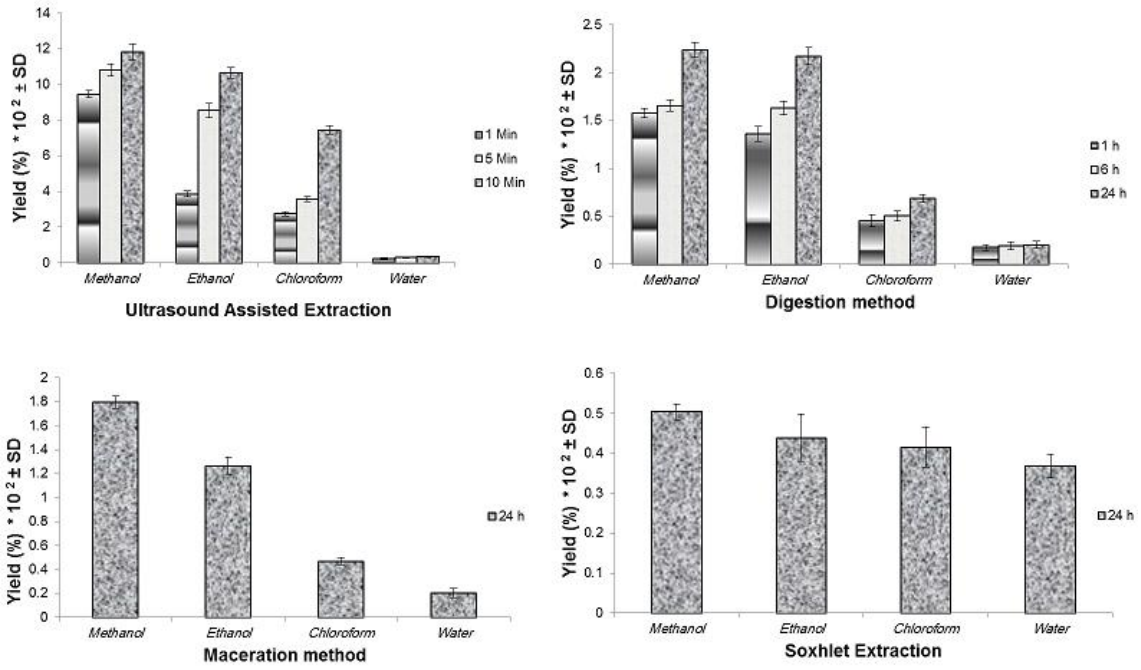

Figure 5. Extraction yield % of different techniques for querectin from *R. sativus*.

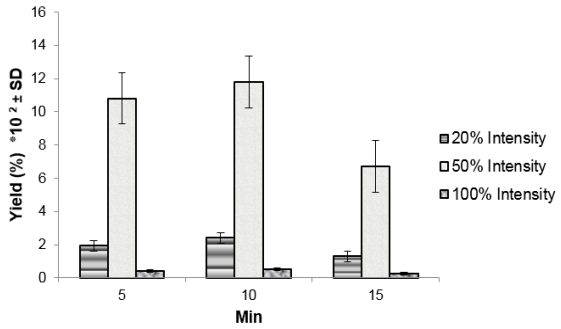

Figure 6. Effects of different times and different ultrasonic apparatus intensities on extraction efficacy of quercetin from *R. sativus* leaves in methanol.

The extraction efficiency above 10 min and also above 50% of ultrasound intensity did not increase, which is the result of destruction of plant material. These results indicate that methanol with 50% of ultrasound intensity in 10 min are the optimized conditions for UAE method.

Conclusion

Due to the polar nature of the phenolic compounds, methanol produced the highest yields in all the cases.

Traditional methods such as maceration, digestion and soxhlet, suffer from long extraction times and also large amount of solvent consumption along with low yields.

As shown by our investigations, UAE was an effective and practical method in order to extract flavonoids such as quercetin from medicinal plants. The superiority of assisted extraction techniques to classic ones are shorter extraction times and using lower amounts of solvents.

The ultrasonic waves promote cavitation in plant cell walls; therefore, this technique is more effective than traditional methods in quercetin extraction from the organic matrix. Despite longer extraction times for maceration, digestion, and soxhlet methods compared to 10 min for UAE technique, the amount of extracted quercetin in UAE was higher than that of digestion, maceration and soxhlet methods.

Acknowledgments

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Conflict of interests

The authors claim that there is no conflict of interest.

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