Middle-East Journal of Scientific Research 17 (2): 252-255, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.17.02.12198

Subcritical Water Extraction of quercetin from *Polygonum hydropiper* L.

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Abstract: The new method for extraction of quercetin from Polygonum hydropiper L. using subcritical water is developed. The method is environmentally friendly and more effective (7.6-times) than traditional extraction methods using expensive and toxic organic solvents. To determine the quantitative and qualitative composition of obtained extracts the high performance liquid chromatography was used.

Key words: Subcritical water · Flavonoid · Quercetin · Extraction · High performance liquid chromatography

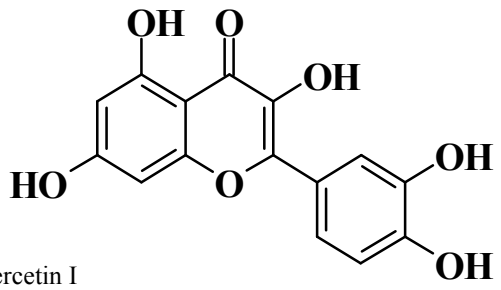
INTRODUCTION

The width of therapeutic action of individual substances with bioflavonoid structure and their mixes, allowed creating a large number of medicinal forms on their basis [1]. The flavonoids have antioxidant and capillary protective (cardiac) effect and also enter into group of vitamin P [2].

Important problem of drugs production on the basis of biologically active substances of plant material is search of new ways of extraction which are environmentally friendly. Subcritical water extraction can be one of such ecologically safe ways. The subcritical water is the water in a liquid state at temperature varying from 100°C to 374°C and pressure higher than that of saturated steams to avoid its transition into the gaseous state [3]. Subcritical water extraction is one of the most recent techniques developed for extracting organic compounds. Advantage of subcritical water is a considerably variability of such important physical characteristics as a constant of dielectric permeability, a superficial tension, a viscosity. At increased temperatures (100°C-374°C) and pressure to 218 atm. physical characteristics of subcritical water decrease and water behaves like weakly polar organic solvent.

The object of the study into present work was a leaves and stems of Polygonum hydropiper L. Polygonum hydropiper L. is an important medicinal plant belongs to the family of Polygonaceae. The whole plant has been found to contain flavones and flavonoid glycosides, such as quercetin galactosides, a sesquiterpene acid, viscosumic acid, oxymethylanthraquinones and polygonic acid [4]. The plant also has some insecticidal properties [5, 6]. The plant also possesses bitter, stimulant, tonic, diuretic, carminative, anthelmintic, haemostatic and lithotripter properties [7]. The whole plant, either on its own or mixed with other herbs used in the treatment of a wide range of ailments including diarrhoea, dyspepsia, itching skin, excessive menstrual bleeding and hemorrhoids [8].

The present work aimed at the development of an ecologically clean method for obtaining bioflavonoid (on an example of quercetin I) containing extracts from Polygonum hydropiper L. using subcritical water for the extraction procedure and studying this method in order to optimize the parameters of the procedure.



Quercetin I

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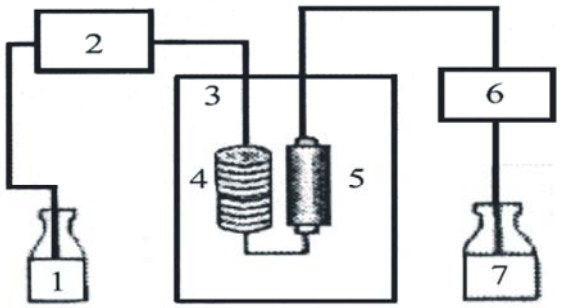

Fig. 1: Scheme of the device used for subcritical water extraction of flavonoids from plant samples: 1. The water tank 2. Pump (Eldex Labs, inc. Menlo Park, CA), 3. The thermostat 4. Spiral of thermostat 5. Cartridge 6. The restrictor (pressure limiter), 7. Capacity to collect the extract.

Experimental: The object of the study was a leaves and a stems of Polygonum hydropiper L. purchased at retail pharmacies "World of herbs" (manufacturer unincorporated business Matyushina L.I., Russia) (State Pharmacopoeia of the XII edition of the Russian Federation).

The extraction by the subcritical water was carried out with the help of the self-made devices (the installation diagram is shown in Figure 1).

The dynamic regime of extraction (flow scheme) was based on water flow through a raw sample at increased temperature and pressure [3], so as to sustain a liquid state of water. Flow extraction was performed in accordance with the previously described protocol. Briefly, a 0.05 g sample of grinded red onion husk (particles 0.5 mm < d < 1.0 mm) was put into an extraction cartridge made of stainless steel of 150mm length and 4.5mm inner diameter. Preliminarily, the extraction cartridge was supplemented with ground glass with the particle size 0.5-1.0 mm (0.5 mL) in order to prevent blocking of the filter inside the communications system. Thereafter, the cartridge was connected to the communications system, placed at a thermostat and heated up to the required temperature at a constant distilled water flow velocity (0.6 mL/min) and pressure (120 atm) that was provided by an HPLC-type pump (Elilex Labs). When the incubation time (30 min) was over, the water flow stopped.

The resulting aqueous extract was treated with chloroform to remove a ballast substances: sequentially extracted with ethyl ether three times with 5 ml ethyl acetate and 3 times with 5 ml. Both extracts were dried over anhydrous sodium sulfate for 12 hours, evaporated to dryness on a rotary evaporator at 40°C and dissolved in 1 ml of methanol. The obtained extracts were filtered and analyzed by chromatographic analysis.

To evaluate the advantages of the proposed method, the subcritical water extraction results were compared with results obtained by conventional methanol extraction of flavonoids.

Traditional extraction of quercetin was performed with organic solvents (80% methanol) [9] in several stages. The methanol extraction is carried out as follows: 0.3 g of air-dried ground plant material was extracted five times with 5 ml of 80% methanol. Water-alcohol extracts were combined and evaporated to 1/3 of the initial volume. To remove chlorophyll and tar the extract was washed with chloroform [10]. The obtained extracts were filtered and analyzed.

For isolation of total flavonoids from methanolic extracts, crude residue was treated sequentially: 1) with diethyl ether 3 times in 5 ml for the extraction aglycones of flavonoids; 2) then with ethyl acetate three times with 5 ml. In the latter case extracted the monoglycoside of flavonoids and the diglycoside of flavonoids.

Both the extract was dried over anhydrous sodium sulfate for 12 hours, evaporated and dissolved in 1 ml methanol for analysis by HPLC chromatography. A typical chromatogram of the extract is shown in Figure 2. The results are presented in Table 1.

The study of the quercetin's concentration in extract was carried out in the reverse-phase HPLC variant using an "Thermo Separation Products" (Model 2000, Thermo Separation Products, Waltham, MA, USA) HPLC equipment. To analyze samples the following chromatography parameters were selected: column Spherisorb ODS-2 C18 2.1 × 150 mm, 3.5 μm; (speed eluent-0.4 ml/min, column temperature-35°C, UV-detector, λ = 353 nm, mobile phase composition: 0.01 M sulfuric acid, acetonitrile, methanol, acetic acid in a ratio of 73:18:4:5; chromatographic assay time-20 min, sample injection amount-20,00 μl).

Quantitative estimation of quercetin concentration in extract was performed by the method of absolute calibration with using a standard quercetin's solutions of differing concentration. Commercial solutions of quercetin in acetonitrile (98.2% purity) (Diaem, Russia) were used as standards. A calibration standard was inserted after every six samples to correct for drift in retention time within a run.

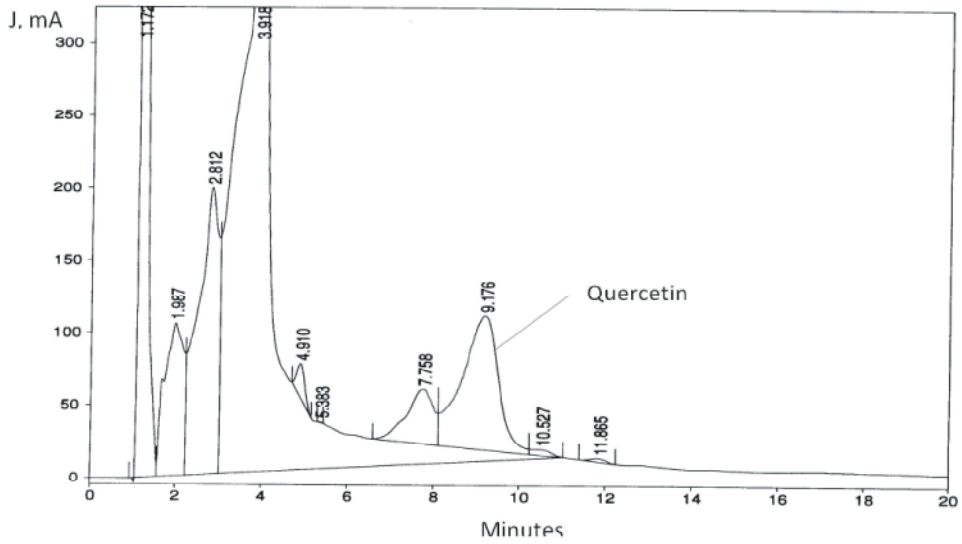

Fig. 2: Chromatogram of quercetin, extracted from Polygonum hydropiper L. leaves by subcritical water.

Table 1: The results of determination of flavonoids and quercetin in the extracts of Polygonum hydropiper L

Solvents	The total amount of extracted flavonoids by HPLC (peak areas, mV*10^8)	Quercetin concentration, µg/g	The share of quercetin extract concerning the method II
Methanol	5,8	43,1	1,0
Subcritical water	4,8	328,0	7,6

RESULTS AND DISCUSSION

The data in Table 1 shows that the amount of quercetin the extracted by subcritical water is in 7.6 higher than yield obtained by the traditional method of extraction. Moreover, this method does not require use of expensive and toxic organic solvents. On the other hand, the data in Table 1 shows that the total amount of flavonoids in the extract obtained in subcritical water is lower than in conventional methanol extraction.

In order to study the causes of such behavior, the extraction by subcritical water was carried out in several temperature ranges. The data in Table 2 show that the amount of quercetin the extracted by water heating before 100 ° C comparable to the amount of quercetin obtained by the traditional method of extraction (44.0 mg / g). That is, there is a full recovery of the free quercetin from the plant matrix. It can be assumed that water heating from 100 ° C to 250 ° C promotes the release of bound quercetin. In addition, this may be due to the hydrolysis of flavonoid glycosides present in plants (such as rutin). This is indirectly supported, in turn, the decrease the amount of flavonoids in extracts subcritical water compared to conventional extraction results (Table 2). As recently shown by the authors on the example of the onion husk

Table 2: Results of quercetin extraction by subcritical water in different temperatures

Number of sample	Temperature, °C	Quercetin concentration, µg/g
1	lower 100	44,0
2	100-150	61,3
3	150-250	222,7

an increase in the temperature of the subcritical water results in a change in the quantitative and qualitative composition of the extracts in the direction of an increase in the amount of aglycones because of acidic hydrolysis [11].

CONCLUSION

An ecologically clean and effective method of extraction of the quercetin from Polygonum hydropiper L. in the medium of subcritical water has been proposed [12-14]. This, in the future, may allow us to develop a technology of selective extraction to obtain extracts with a desired ratio of glycosides and aglycones. The method is environmentally friendly and more effective (7.6-times) than traditional extraction methods using expensive and toxic organic solvents. However, this process is three to four times faster and does not require use of expensive and toxic organic solvents.

ACKNOWLEDGEMENTS

This work was supported by grant of the Southern Federal University ([1] 213.01-24/2013-48) and grants of Russian Foundation for Basic Research [1] 13-03-01318, 13-03-12271.

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