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Ficaria verna Huds. extracts and their β-cyclodextrin supramolecular systems

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Abstract

Background: Obtaining new pharmaceutical materials with enhanced properties by using natural compounds and environment-friendly methods is a continuous goal for scientists. *Ficaria verna* Huds. is a widespread perennial plant with applications in the treat of haemorrhoids and to cure piles; it has also anti-inflammatory, astringent, and antibiotic properties. The goal of the present study is the obtaining and characterization of new *F. verna* extract/β-cyclodextrin complexes by using only natural compounds, solvents, and environment-friendly methods in order to increase the quality and acceptability versus toxicity indicator. Thus, the flavonoid content (as quercetin) of *Ficaria verna* Huds. flowers and leaves from the West side of Romania was determined and correlated with their antioxidant activity. Further, the possibility of obtaining β-cyclodextrin supramolecular systems was studied.

Results: *F. verna* flowers and leaves extracts were obtained by semi-continuous solid-liquid extraction. The raw concentrated extract was spectrophotometrically analyzed in order to quantify the flavonoids from plant parts and to evaluate the antioxidant activity of these extracts. The *F. verna* extracts were used for obtaining β-cyclodextrin complexes; these were analyzed by scanning electron microscopy and Karl Fischer water titration; spectrophotometry was used in order to quantifying the flavonoids and evaluates the antioxidant activity. A higher concentration of flavonoids of 0.5% was determined in complexes obtained by crystallisation method, while only a half of this value was calculated for kneading method. The antioxidant activity of these complexes was correlated with the flavonoid content and this parameter reveals possible controlled release properties.

Conclusions: The flavonoid content of *F. verna* Huds. from the West side of Romania (Banat county) is approximately the same in flowers and leaves, being situated at a medium value among other studies. β-Cyclodextrin complexes of *F. verna* extracts are obtained with lower yields by crystallisation than kneading methods, but the flavonoids (as quercetin) are better encapsulated in the first case most probably due to the possibility to attain the *host-guest* equilibrium in the slower crystallisation process. *F. verna* extracts and their β-cyclodextrin complexes have antioxidant activity even at very low concentrations and could be used in proper and valuable pharmaceutical formulations with enhanced bioactivity.

Keywords: *Ficaria verna* Huds, β-cyclodextrin, Supramolecular systems, Inclusion compounds, Molecular encapsulation, Micro- and nanoparticles, Flavonoids, Quercetin, Antioxidant activity

Background

Ficaria verna Huds. (lesser celandine, fig buttercup) is a widespread perennial plant which is found throughout Europe, West Asia, and North America, being sometimes called "the spring messenger" due to the blossoming period starting from March. It is also called "an invasive" plant in North America due to the use of these species for specific restoration-preservation processes in native habitats [1-3]. The name is derived from the Latin *ficus*, which means fig, due to the resembling of the *F. verna* roots with these fruits. It belongs to Ranunculaceae family, comprises of 59 genera and about 1900 species [4,5].

In folk medicine *F. verna* was used to treat haemorrhoids and to cure piles, being known also as pilewort. Other traditional applications were in anti-inflammatory, astringent, antibiotic, and anti-haemorrhagic treatments [4,6,7]. *F. verna* reveals a high content of vitamin C,

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which confer anti-scorbutic activity. The consumption of the plant before blossoming in salads, soups, or other foodstuffs, combats the fatigue which appears in spring time [1]. Extracts and tinctures of *F. verna* are used especially in external treatments, but also in some oral cavity infections [6-8].

The *F. verna* plant contains saponosides (having oleanolic acid, ficaric acid, ficarin, anemonin as aglycone moiety), flavonoids, vitamin C, minerals, and ranunculin. From the flavonoid class the most important are kaempferol and quercetin with their 3-*O*- and 7-*O*-glycosides (e.g. rutinoside), as well as 8-*C*-glycosidic derivatives (vitexin, orientin, vitexin-2"-*O*-glucoside) [1,4,6,7].

Flavonoids such as quercetin and rutin are responsible for the anti-inflammatory and other biological activities of *F. verna* extracts, but these polyphenolic compounds are not very stable due to their susceptibility to oxidation. In order to protect them against environmental degradation factors (air/oxygen, light, humidity) and to obtain formulations with controlled release properties molecular encapsulation can be used. Some of the better matrices for molecular encapsulation (*host-guest* supramolecular systems or inclusion compounds) are cyclodextrins, which are naturally occurring cyclooligosaccharides mainly containing 6-8 glucopyranose moieties (α-, β-, and γ-cyclodextrin, αCD, βCD, and γCD, respectively), and having a hydrophobic inner cavity with a capacity to enclose (partial or total) small organic molecules [9-14]. The presence of primary and secondary hydroxyl groups to the exterior increase the water solubility of these compounds and the corresponding *host-guest* complexes. Some studies on the complexation and properties of flavonoids and other similar derivatives in cyclodextrins were done [13,15-17].

This paper continues our research in the cyclodextrin field [15,16,18-30] and presents a study among the content of flavonoids and other similar compounds (as quercetin) of *F. verna* Huds. flowers and leaves from the West side of Romania, as well as the β-cyclodextrin complexation of the corresponding extracts. The correlation of antioxidant activity of these extracts with the flavonoid content was also studied. Further, the possibility of obtaining β-cyclodextrin supramolecular systems containing bioactive compounds such as flavonoids from *F. verna* (with potential enhanced protecting capacity and controlled release properties) and characterization of them were studied (Figure 1). All methods and compounds used in the obtaining of raw extracts and β-cyclodextrin complexes were the most simplest and from natural sources in order to obtain a very high quality and acceptability versus toxicity indicator. β-Cyclodextrin was the appropriate host molecule for encapsulation due to the following: the cheapest natural cyclodextrin which can be used in "green" complexation of natural compounds. Other cyclodextrins with higher water solubility such as hydroxypropyl-cyclodextrins, randomly methylated cyclodextrins, sulfobutylether-β-cyclodextrin, or branched cyclodextrins are semi-synthetically modified cyclodextrins and were not the goal of this study. Furthermore, from the methods used in cyclodextrin complexation only two were studied (both non-expensive and economically appropriate for further applications): the simplest kneading method (mixing the cyclodextrin and bioactive extract in the presence of a small amount of water-ethanol solvent, drying at normal temperature, and grinding) and the controlled crystallisation method from ethanol-water system; the last method provide the best cyclodextrin complex due to the possibility to attain the association-dissociation equilibrium (this cannot be attained by the spray-drying method, which could be used especially at industrial level for a good yield).

Materials and methods

Materials

All *F. verna* Huds. plants were collected from the West side of Romania (Banat county) in April 2010, when these plants were in blossoming period; the raw flowers and leaves were separated and stored in sealed flasks at -20°C until extraction. In the extraction and complexation processes ethanol 96% (v/v) from Chimopar (Bucharest), was used. Quercetin and β-cyclodextrin (purity > 98%) were achieved from Fluka Chemie AG and DPPH (2,2-diphenyl-1-picryl-hydrazyl, 99% purity) was obtained from Sigma-Aldrich. Titrant 5 apura®, Solvent apura®, and Water standard 1% apura®, used for two-component Karl Fischer water titration, were purchased from Merck&Co., Inc.

Obtaining of *Ficaria verna* Huds. extracts

F. verna Huds. extracts were obtained by semi-continuous solid-liquid extraction by using a Soxhlet apparatus of 100 mL. The raw *F. verna* flowers or leaves were finely grounded in a mortar and the weighted samples were put in the extraction device cellulose cartridge. In the extraction flask 150 mL ethanol 96% was added as well as a boiling regulator; the extraction flask was heated on a water bath and three to five extraction cycles were performed for every sample (until the extract from the last cycle do not reveal significant absorbance in the spectrophotometric analysis). The raw extract was concentrated in a rotary evaporator in vacuum (Vacuum Rotary Evaporator type 350, Unipan, Poland) at approximate of 1:5 from the initial volume (Table 1). These concentrated extracts were analyzed and used for obtaining β-cyclodextrin complexes and for antioxidant activity evaluation. All extracts were performed in triplicate [see Additional file 1].

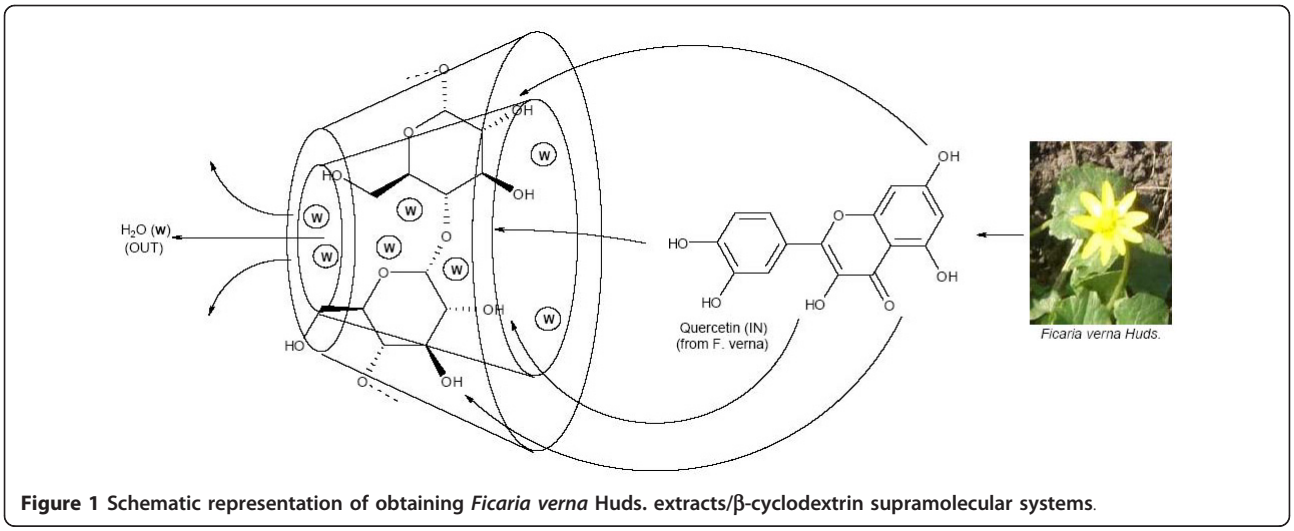

Figure 1 Schematic representation of obtaining *Ficaria verna* Huds. extracts/β-cyclodextrin supramolecular systems.

Obtaining of *Ficaria verna* Huds. extract/β-cyclodextrin complexes by crystallisation method

Obtaining of new supramolecular systems containing *F. verna* extracts were realized by complexation in natural β-cyclodextrin. The first method used for obtaining these complexes was the controlled crystallisation from the ethanol-water solution. The study of the complexation parameters were studied and published elsewhere for similar supramolecular systems [23,27]; only optimal parameter values were used in this study. Thus, 0.5 mmoles of β-cyclodextrin was dissolved in 4 mL distilled water in a 10 mL complexation reactor, equipped with a thermostatic jacket and a dropping funnel. The βCD solution was heated to 50°C and a volume of the *F. verna* concentrated extract corresponding to a molar ratio of 4:1 between βCD and flavonoids (expressed as quercetin) was added to the βCD solution under magnetic stirring in 15 minutes. The suspension was stirred at 50°C for another 15 minutes and after that the slow controlled cooling of the reactor was started, with a cooling rate of 7.5°C/hour. The suspension of complex crystals obtained after 4 hours was stored over night at 4°C in a refrigerator. The suspension was filtered in vacuum, washed with 1 mL 96% ethanol and dried in exicator at 40°C until constant mass. The complex recovering yield was calculated as the ratio between the complex mass and the sum of the starting materials (βCD and flavonoids as quercetin). The obtained *F. verna* extract/βCD complex was analyzed by scanning electron microscopy, Karl Fischer water titration, and spectrophotometry in order to evaluate the flavonoid content and antioxidant activity [see Additional file 1].

Obtaining of *Ficaria verna* Huds. extract/β-cyclodextrin complexes by kneading method

The *F. verna* extract/βCD complexes were obtained also by using the kneading method [31,32]. βCD and *F. verna* extract in the above mentioned quantities were kneaded for 15 minutes in a mortar in the presence of a small volume of water (a ratio of 2:1 between extract and water, by volume) at the constant temperature of 50°C. The viscous mixture was filtered in vacuum, washed with 1 mL 96% ethanol (in order to remove the non-complexed compounds-flavonoids and partially β-cyclodextrin), and dried until constant mass. The cyclodextrin complex was grinded and sieved through 0.25 mm sieve. The resulted βCD complexes were analyzed in the same manner as in the case of complexes obtained by crystallisation method [see Additional file 1].

Table 1 Conditions and results for obtaining *Ficaria verna* Huds. ethanolic extracts, "EE_Fv" ("Fl"-flower, "Lf"-leaf); "a-c"-replicates

No	Code	Sample weight (g)	Volume of ethanol (mL)	Extraction time (min)	No of cycles	Volume of extract (mL)
1	EE_Fv_Lf_a	20.0	150	127	4	37
2	EE_Fv_Lf_b	20.0	150	210	3	43
3	EE_Fv_Lf_c	20.0	150	150	4	37
4	EE_Fv_Fl_a	15.0	150	152	5	36
5	EE_Fv_Fl_b	15.0	150	177	3	36
6	EE_Fv_Fl_c	15.0	150	125	5	40

Karl Fischer water titration (KFT)

Karl Fischer water titration of βCD complexes and commercial βCD was carried out by using a Karl Fischer 701 Titrando apparatus from Metrohm; a Metrohm 10 dosing system and 703 Ti Stand mixing systems were also used. The two-component technique was used for water determination (Component 1: Titrant 5 apura® and Component 2: Solvent apura®). The titer of component 1 was performed by using Water standard 1% apura®, standard for volumetric Karl Fisher titration (a titer of 4.4849 mg/mL was determined). The sample amount was ~0.05 g. The method parameters were: *I* (pol) of 50 μA, end point and dynamics at 250 mV, maximum rate of 5 mL/min, drift was used as stop criterion, with a stop drift of 15 μL/min. The extraction time was 300 s. All determinations were done at least in triplicate [see Additional file 2].

Scanning electron microscopy (SEM) analysis

For morphological and dimensional evaluation of the *F. verna* extracts/βCD complexes the scanning electron microscopy (SEM) technique was used. An Inspect S SEM apparatus, with a voltage of 25 kV, 300× to 3000× magnitude level, and focusing of 10-14.1 mm was used. Prior to examination, samples were gold sputter-coated, to render them electrically conductive.

Spectrophotometric analysis and antioxidant activity evaluation

The presence of flavonoids containing phenolic OH groups (such as quercetin) confers to the *Ficaria verna* extracts antioxidant character. The antioxidant activity of *F. verna* extracts as well as of their βCD complexes was evaluated by using DPPH spectrophotometric method (UV-VIS CamSpec 501 apparatus); the acquisition and handling of the data were realized with the UV-vis Analyst program, ver. 4.67. Thus, the sample cuvette contains 2 mL ethanol (96%, v/v), 0.5 mL *F. verna* extract (undiluted or diluted), quercetin or βCD complex solutions, and 0.5 mL DPPH ethanolic solution (concentration of 1 mM). The spectrophotometric analysis was realized at 517 nm for 15 minutes. Ethanol was used as reference solvent. The same apparatus was used in order to quantify the flavonoid content of *F. verna* extracts and their βCD complexes (as quercetin) [see Additional file 1].

Results and discussion

Obtaining and analysis of *Ficaria verna* Huds. extracts

Overall biocompound concentration of *F. verna* extracts was evaluated by means of flavonoid concentration (expressed as quercetin concentration, one of the main flavonoid compounds from these plants). Eight quercetin standard solutions with concentrations of 0.1 mg/100 mL to 1.0 mg/100 mL were analyzed spectrophotometrically (maximum absorbance of 256 nm was recorded) in order to quantify these compounds with biological activity. The equation of the calibration curve is presented below (r^2 = 0.98).

$$Absorbance\,(@256\ \text{nm}) = 0.769\,(\pm 0.021) \times Concentration\,(\text{mg}/100\ \text{mL})$$

By using this calibration curve and the spectrophotometric analysis of *F. verna* Huds. extracts (one hundred fold dilutions) the flavonoid concentration (expressed as quercetin) could be evaluated. A high concentration of flavonoids was identified in flowers, 202.0 ± 16.4 mg/100 g raw sample, as well as in leaves (223.3 ± 33.0 mg/100 g raw samples) (Table 2) [see Additional file 1].

Obtaining and analysis of *Ficaria verna* Huds. extract/β-cyclodextrin supramolecular systems

F. verna extract/βCD complexes were obtained by using two methods: controlled crystallisation from ethanol-water solution and kneading methods, both in a molar ratio of 1:4 for the calculated flavonoid content (as quercetin) and βCD. In the case of crystallisation method the complex recovering yield was 59.3 ± 0.3% for the *F. verna* flower extract/βCD complex and 49.0 ± 2.6% for the *F. verna* leaf extract/βCD complex. The second method conduct to a better complex recovering yield, higher for the *F. verna* flower/βCD complex (91.7 ± 4.3%) and with 5% lower for the *F. verna* leaf/βCD complex (86.9 ± 0.4%). These differences between the recovering yields are due to the water solubility of βCD and the corresponding *F. verna* extract/βCD complexes; in the crystallisation method the association-dissociation of the biocompounds-βCD equilibrium tend to be attained. This equilibrium depends on the water solubility of βCD and its complexes; further, the separation process (filtration, washing, and drying) allow to partially loose βCD and complexes. In the case of kneading method only some volatile substances and ethanol-soluble compounds from extracts as well as the complex recovering process influences the global yield. As a result, the recovering yield in the kneading method is 32-38% higher than in the case of crystallisation method.

The analysis of the βCD complexes was performed by using scanning electron microscopy (SEM) in order to evaluate the morphology and approximate dimensions of micro/nanoparticles. Karl Fischer titration (KFT) was used in order to establish the water content of βCD complexes. Finally, the spectrophotometric analysis was used for evaluation of the encapsulated biocompounds and for antioxidant activity evaluation of extracts and their βCD complexes (see below).

SEM analysis was performed at magnitudes of 300-3000× and reveals that the commercial βCD (which was

Table 2 Flavonoid concentrations (expressed as quercetin) from the *Ficaria verna* Huds. ethanolic extracts, "EE_Fv", and plant parts ("Fl"-flower, "Lf"-leaf); "a-c"-replicates

No	Code	Dilution factor	Absorbance (@256 nm)	Flavonoid concentration (in extract) (mg/100 mL)	Flavonoid concentration (in raw plant) (mg/100 g)
1a	EE_Fv_Fl_a	100	0.910	118.4	219.0
1b	EE_Fv_Fl_b	100	0.666	86.6	186.2
1c	EE_Fv_Fl_c	100	0.835	108.6	200.9
1	**EE_Fv_Fl**			**104.5 ± 16.3**	**202.0 ± 16.4**
2a	EE_Fv_Lf_a	100	0.835	108.6	260.6
2b	EE_Fv_Lf_b	100	0.633	82.4	197.7
2c	EE_Fv_Lf_c	100	0.610	79.4	211.7
2	**EE_Fv_Lf**			**90.1 ± 16.1**	**223.3 ± 33.0**

used in complexation) has crystals with rhomboidal shapes; generally, the sides of the rhomboid vary in a large range, being lower than 50 μm (Figure 2a). In the case of *F. verna* extract/βCD complexes obtained by crystallisation method the crystals are prismatic, with mean dimensions of ~20 μm (Figures 2b and 2c). No uniformity (dimensions and shapes) of particles obtained by kneading method was observed, the maximum size of these crystals being 100 μm (Figures 2e and 2f).

Water analysis of cyclodextrin complexes is an important tool for evaluation of the quality of the complexation process: if the biocompound-cyclodextrin interaction is appropriate the water molecules from the inner cavity of cyclodextrin are replaced and the final water content is low (Figure 1); otherwise, these water molecules remain in complexes and a high water content of cyclodextrin complexes could be determined. Taking into account these observations, the water content of *F. verna* extract/βCD complexes was evaluated. The best method used for this reason is Karl Fischer titration method, which allows to evaluate only the water content; this is the best method for water determination, in comparison with other methods used for water/humidity evaluation (e.g. thermogravimetric analysis, which determines all volatiles, including water [15,20-22,30]). Thus, the water content of *F. verna*/βCD complexes were lower than in the case of commercial βCD; the starting cyclodextrin has a water content of 15.3 ± 0.15%, while all βCD complexes have lower water content (differences up to 5%). A significant difference exists also between the water content of βCD complexes obtained by the above mentioned methods: a water content of 11.3 ± 0.02% was obtained in the case of *F. verna* flower extract/βCD complex obtained by crystallisation method, while this value was higher in the case of the same complex obtained by kneading method (12.95 ± 0.59%); similar aspect was observed in the case of *F. verna* leaf extract/βCD complexes (10.91 ± 0.17% for crystallisation method and 13.24 ± 0.16% for kneading method) (Table 3). A lower content of water in cyclodextrin complexes indicates that the inclusion compound was obtained due to a partial replacing of water molecules by *guest* compounds-i.e. flavonoids and other bioactive molecules from *F. verna* extracts. The types of the water molecules could be also evaluated (qualitatively) by means of the slope of the correlation curves for the pseudolinear variation of the titration volume, normalized to the weight of the sample, in time (*V/m* vs. *Time* titration curve, Figure 3). Thus, the "surface water" molecules (water molecules from the crystal surface and which are located between cyclodextrin molecules) react with a higher rate in the first time interval from the titration curve (up to 30-40 s), while the "strong-bonded" water molecules (water molecules from the inner cavity of cyclodextrins) react with a lower rate in a time interval of approximate 70 s (up to 100 s); the last interval have a "normal slope" for the "standby" of the KFT apparatus [see Additional file 2].

The concentration of cyclodextrin encapsulated compounds from *F. verna* extracts (principally flavonoids expressed as quercetin) was evaluated by using the same spectrophotometric method. This determination was performed on 2% aqueous solutions of *F. verna* extract/βCD complexes, by using the same calibration curve for quercetin. The concentration of encapsulated biocompounds from *F. verna* extracts were higher in the case of βCD complexes obtained by crystallisation method (0.50 ± 0.06% for the case of flower extracts and 0.46 ± 0.23% for the case of leaves extract), while this concentration was almost at a half in the case of kneading method (0.28 ± 0.01% for the case of flower extracts and 0.25 ± 0.04% for the case of leaves extract) [see Additional file 1]. Theoretically, the molar ratio between biocompounds from *F. verna* extracts (expressed as quercetin) and cyclodextrin was 1:4, which could conduct to a percent concentration of 4.3% flavonoid in cyclodextrin complexes, but the higher hydrophilic properties of flavonoids demonstrate that the hydrophobic interaction with the inner cavity of cyclodextrin is poor; this observation is demonstrated by the final concentration of these compounds in the studied complexes: ~0.5% by crystallisation method and a half for kneading method. This difference can be

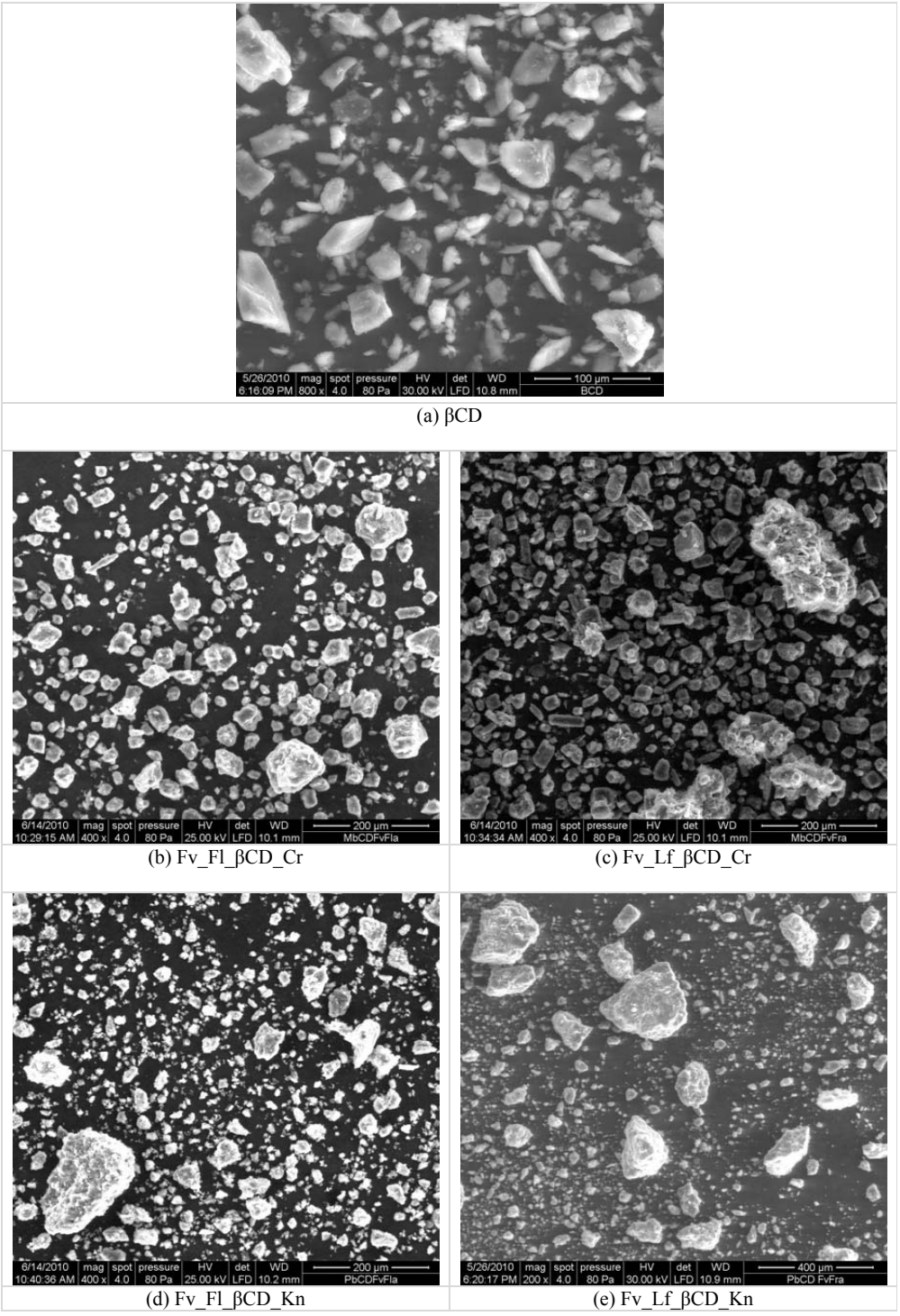

Figure 2 SEM analysis of βCD and its complexes with *Ficaria verna* extracts (flower and leaf).

explained by the possibility to attain the flavonoid-cyclodextrin equilibrium, which is better performed in the crystallisation method (slow crystallisation of the complex), while a high quantity of biocompounds (uncomplexed ones) were removed.

Antioxidant activity of *Ficaria verna* Huds. extracts and their β-cyclodextrin complexes

Antioxidant activity of *F. verna* extracts and their β-cyclodextrin complexes was evaluated by using the radical scavenging property of DPPH (2,2-diphenyl-1-pycryl-

Table 3 Water content of *Ficaria verna* Huds. extract/β-cyclodextrin complexes and commercial β-cyclodextrin, determined by Karl Fischer titration ("Cr"-crystallisation method, "Kn"-kneading method, "Fl"-flower, "Lf"-leaf; "a-d"-replicates)

No	Code	Sample weight (g)	Volume of the titrant (mL)	Water content (%)
1a	βCD_a	0.0453	1.547	15.32
1b	βCD_b	0.0322	1.084	15.10
1c	βCD_c	0.0294	1.009	15.39
1	**βCD**			**15.27 ± 0.15**
2a	Fv_Fl_βCD_Cr_a	0.0512	1.29	11.30
2b	Fv_Fl_βCD_Cr_b	0.0503	1.27	11.32
2c	Fv_Fl_βCD_Cr_c	0.0508	1.284	11.34
2	**Fv_Fl_βCD_Cr**			**11.32 ± 0.02**
3a	Fv_Lf_βCD_Cr_a	0.0498	1.231	11.09
3b	Fv_Lf_βCD_Cr_b	0.0498	1.224	11.02
3c	Fv_Lf_βCD_Cr_c	0.051	1.222	10.75
3d	Fv_Lf_βCD_Cr_d	0.0514	1.238	10.80
3	**Fv_Lf_βCD_Cr**			**10.91 ± 0.17**
4a	Fv_Fl_βCD_Kn_a	0.0417	1.255	13.50
4b	Fv_Fl_βCD_Kn_b	0.0506	1.39	12.32
4c	Fv_Fl_βCD_Kn_c	0.0507	1.474	13.04
4	**Fv_Fl_βCD_Kn**			**12.95 ± 0.59**
5a	Fv_Lf_βCD_Kn_a	0.0506	1.512	13.40
5b	Fv_Lf_βCD_Kn_b	0.0477	1.392	13.09
5c	Fv_Lf_βCD_Kn_c	0.0547	1.613	13.23
5	**Fv_Lf_βCD_Kn**			**13.24 ± 0.16**

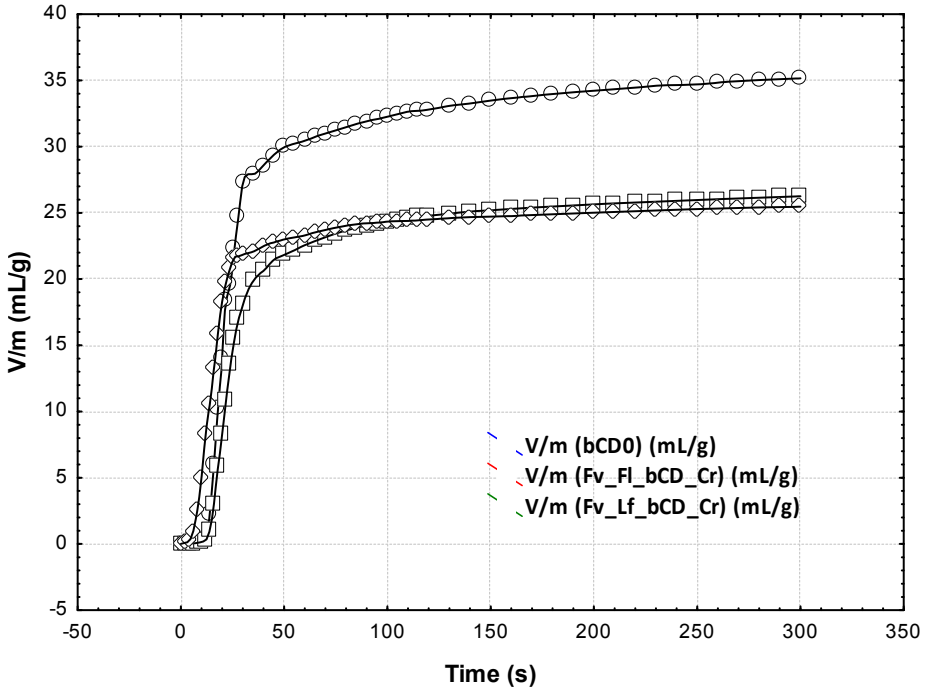

Figure 3 Karl Fischer titration analysis for the *Ficaria verna* extracts/β-cyclodextrin complexes (obtained by crystallisation method) and commercial β-cyclodextrin (upper curve).

hydrazyl). Generally, antioxidants act as radical trapping compounds (i.e. peroxy radicals resulted by auto-oxidation of fatty acids), and the antioxidant activity could be evaluated by using such as radicals; DPPH is one of the most stable organic radical, which can be used as radical trapping.

A large class of antioxidants (even synthetic or natural) is hindered phenols; these compounds easily react with free radicals (i.e. peroxy radicals) and generate phenoxy radicals. As a consequence the neutralization of the first dangerous ones take place. Phenoxy radicals resulted from the hindered phenol antioxidant compounds regenerate the starting phenols and also furnish quinone or quinone methides, which have limited stability and react to yield a complex mixture of products. The role of free radicals could be taken by DPPH and the overall antioxidant activity could be evaluated spectrophotometrically due to the fact that DPPH have a maximum absorbance at 517 nm. The above mentioned reaction can be monitored by measuring this absorbance in the presence of samples containing antioxidants.

The antioxidant activity of samples containing flavonoids was calculated according to the following equation:

$$AA\,(\%) = 100 - \left[Abs.\,(t)\,/Abs.\,(t = 0) \times 100\right]$$

Where AA represents the antioxidant activity, $Abs.(t)$ represents the absorbance of the reaction mixture (solution of flavonoid sample and DPPH) at the time t (measured at the wavelength of 517 nm), while $Abs.(t = 0)$ represents the initial absorbance of this mixture in the same conditions. A higher value of AA indicates a higher antioxidant activity of the sample.

Four quercetin standard solutions with concentrations of 1600 μM, 160 μM, 16 μM, and 1.6 μM were prepared and analyzed spectrophotometrically for evaluation the antioxidant activity. Antioxidant activities of these standard samples, evaluated according to the above mentioned equation after 300 s in the presence of DPPH, are 89% for the most concentrated solution, followed by 49% for the second one. The last two quercetin solutions have lower but important antioxidant activities (9% and 5%, respectively).

The antioxidant activity was evaluated in the same manner for the *F. verna* extracts and their βCD complexes, for both raw and diluted samples (5- and 25-fold dilutions). Thus, all studied samples (extracts and aqueous βCD complex solutions) show antioxidant activity by means of decreasing the absorbance of the sample-DPPH mixture at 517 nm; this decreasing is more significant in the case of undiluted samples and remains important also in the case of diluted ones. The same aspect was observed in the case of the corresponding *F. verna*/βCD complexes (2% aqueous solution), but less significant (resembling to 25-fold diluted extracts) due to the concentration of the bioactive compounds. The antioxidant activity of the 25-fold diluted *F. verna* extract (both flower and leaf) is approximately the same: 13.2 ± 2.5% and 12.9 ± 2.8% for flower and leaf extracts, respectively. These values are placed between the antioxidant activity of quercetin standard solutions with concentrations of 160 μM and 16 μM. The antioxidant activity of the corresponding βCD complexes was little bit lower: 5.1 ± 1.2% and 5.6 ± 2.8% for the *F. verna* flower and leaf/βCD complexes obtained by crystallisation method; in the case of *F. verna* flower and leaf/βCD complexes obtained by kneading method these values were 4.5 ± 2.5% and 7.9 ± 1.2%. Higher antioxidant activity values were obtained in the case of *F. verna* leaf extract/βCD complexes for both complexation methods (5.6% for crystallisation method and 7.9% for kneading method) [see Additional file 1]. These values resemble with the quercetin standard solutions with concentrations of 160 μM and 16 μM.

An important parameter in antioxidant activity evaluation is the DPPH reaction rate on various time ranges in the presence of antioxidant compounds. The DPPH standard curve was calculated spectrophotometrically at 517 nm (r^2 = 0.99):

$$Absorbance\,(@517\ \mathrm{nm}) = 10960\,(\pm 50) \times Concentration\,(\mu\mathrm{M}) + 25\,(\pm 1)$$

This equation was used for calculation of the momentarily DPPH concentration in the presence of antioxidant compounds and further for determination of the mean DPPH rate on different pseudolinear ranges from the *Concentration (DPPH, μM)* vs. *Time (s)* antioxidant evaluation plot. This mean reaction rate was calculated by using the following equation:

$$v_{mean} = -[\Delta c_{DPPH}/\Delta t]\ \ (\mu\mathrm{M/s})$$

Where Δc_{DPPH} is the variation of the DPPH concentration on the studied range and Δt is the time interval. The rate results from the slope value of the *Concentration* vs. *Time* linear correlation for the studied time range. Thus, three time ranges were identified for antioxidant activity evaluation time of maximum 900 s: v_1 for the time range of 0-50 s, v_2 for the time range of 50-300 s, and v_3 for the time range of 300-900 s. Thus, the mean DPPH reaction rate of the *F. verna* flower 25-fold diluted extracts decrease from 0.3 μM/s for the first interval to 0.05 μM/s for the second one, and finally to 0.01 μM/s for the last interval. Little bit lower decrease was observed in the case of *F. verna* leaf extracts, at the same dilution (from 0.18 μM/s to 0.03 μM/s, and finally to 0.01 μM/s). In the case of cyclodextrin complexes this decrease of the DPPH reaction rate is lower than in the case of non-encapsulated *F. verna* extracts: in the first and second intervals the DPPH reaction rate in the presence of cyclodextrin

complex solutions is 5-fold and 3-fold lower than in the case of non-encapsulated flower and leaf samples, respectively. In the last interval only 2-fold lower DPPH reaction rate was calculated for the cyclodextrin complexes in comparison with non-encapsulated extracts [see Additional file 1]. Further, the ratios between the mean DPPH reaction rates from different time ranges could indicate the controlled release properties of cyclodextrin complexes (further studies will be needed). This aspect is evident in the case of complexes obtained by crystallisation method, where the ratio between v_2 and v_3 is lower than in the case of non-encapsulated samples (v_2/v_3 of 5.6 and 4.5 for non-encapsulated and βCD encapsulated *F. verna* flower extracts, 3.2 and 2.8 for the corresponding leaf extracts, respectively). These observations reveal also that the *host-guest* inclusion process is better achieved by using the crystallisation method in comparison with kneading method.

Conclusions

The following conclusions among the extraction and analysis of bioactive compounds from *Ficaria verna* Huds. species as well as the cyclodextrin complexation and antioxidant activity evaluation of non-encapsulated and encapsulated extracts can be drawn: (1) the content of flavonoids and other resembling compounds (expressed as quercetin) of *Ficaria verna* Huds. from the West side of Romania (Banat county) is approximately the same in flowers and leaves (harvested in blossoming period), being situated at a medium value among other studies (1-3.5 mg/g) [4,6,7]; (2) β-cyclodextrin complexes of *F. verna* extracts are obtained with lower yields by crystallisation than kneading methods, but the flavonoids (as quercetin) are better encapsulated in the first case most probably due to the possibility to attain the *host-guest* equilibrium in the slower crystallisation process; (3) water content of cyclodextrin complexes is an indirect parameter which demonstrate the quality of the *host-guest* interaction-the water concentration in cyclodextrin complexes is lower in comparison with the commercial cyclodextrin due to the replacing of water molecules by more hydrophobic bioactive flavonoids from *F. verna* extracts. However, the differences between water content values of cyclodextrin and complexes are not very high due to the fact that flavonoids and especially flavonosides or saponins (which are evaluated as quercetin) have a lower hydrophobicity (the explanation is the great number of hydroxyl groups); as a result, the van der Waals interactions with the inner cavity of cyclodextrins are relatively poor. These observations correlates with the flavonoid content of complexes obtained by these two methods; (4) both *F. verna* extracts and their β-cyclodextrin complexes have antioxidant activity even at very low concentrations. Antioxidant activity (which is related with the above mentioned biological activities) can be better monitored by means of the reaction rate of the model radical species.

Additional material

Additional file 1: Spectrophotometric analysis. In this additional file the UV-vis analyses of *Ficaria verna* extracts and β-cyclodextrin complexes as well as their kinetic studies by DPPH method are presented; further, the *AA/Concentration (DPPH)* vs. *Time* variations, including mean DPPH reaction rates for all samples studied are also shown. These data supports all considerations presented in the manuscript.

Additional file 2: Karl Fischer water titration. In this additional file the *V/m* vs. *Time* (titrant volume, normalized to sample weight versus time of titration) plots for all *Ficaria verna* extracts/β-cyclodextrin complexes from KFT analysis were presented; they supports all considerations presented in the manuscript.

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Authors' contributions
NGH carried out all experiments and prepared the final manuscript.

Competing interests
The author declares that she has no competing interests.

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References

1. Woodward SL, Quinn JA: *Encyclopedia of Invasive Species. From Africanized Honey Bees to Zebra Mussels* Santa Barbara: Greenwood Publishing Group; 2011.
2. Taylor K, Markham B: **Biologcal Flora of the British Isles.** *J Ecol* 1978, **66**:1011-1031.
3. Swearingen J, Reshetiloff K, Slattery B, Zwicker S: *Lesser Celandine. Plant Invaders of Mid-Atlantic Natural Areas* Washington DC: National Park Service and U.S. Fish & Wildlife Service; 2002.
4. Tomczyk M, Gudej J, Sochacki M: **Flavonoids from *Ficaria vern* Huds.** *Z Naturforsch* 2002, **57c**:440-444.
5. Pigg KB, Devore ML: **Paleoactaea gen. nov. (Ranunculaceae) Fruits from the Paleogene of North Dakota and the London Clay.** *Am J Bot* 2005, **92**:1650-1659.
6. Gudej J, Tomczyk M: **Polyphenolic Compounds from flowers of *Ficaria vern* Huds.** *Acta Poloniae Pharmaceutica-Drug Research* 1999, **56**:475-476.
7. Tomczyk M, Gudej J: **Quantitative Analysis of flavonoids in the flowers and leaves of *Ficaria vern* Huds.** *Z Naturforsch* 2003, **58c**:762-764.
8. Gudej J, Tomczyk M: **Determination of flavonoids, tannins and ellagic acid in leaves from *Rubu* L. Species.** *Arch Pharm Res* 2004, **27**:1114-1119.
9. Costescu C, Corpaş L, Hădărugă NG, Hădărugă DI, Gârban Z: **Cyclodextrins and small unilamellar liposomes: a comparative theoretical approach.** *Studia Universitatis UBB, Seria Chemia* 2011, **56**:83-88.
10. Brewster ME, Loftsson T: **Cyclodextrins as pharmaceutical solubilizers.** *Adv Drug Deliv Rev* 2007, **59**:645-666.
11. Steffen A, Apostolakis J: **On the ease of predicting the thermodynamic properties of β-cyclodextrin inclusion complexes.** *Chemistry Central Journal* 2007, **1**:29[http://dx.doi.org/10.1186/1752-153X-6-16].
12. Szejtli J, Szente L: **Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins.** *Eur J Pharm Biopharm* 2005, **61**:115-125.

13. Jullian C, Moyano L, Yänez C, Olea Azar C: **Complexation of quercetin with three kinds of cyclodextrins: an antioxidant study.** *Spectrochimica Acta Part A* 2007, **67**:230-234.
14. Haiyun D, Jianbin C, Guomei Z, Shaomin S, Jinhao P: **Preparation and spectral investigation on inclusion complex of β-cyclodextrin with rutin.** *Spectrochimica Acta Part A* 2003, **59**:3421-3429.
15. Hădărugă DI, Hădărugă NG, Bandur G, Isengard H-D: **Water content of flavonoid/cyclodextrin nanoparticles: relationship with the structural descriptors of biologically active compounds.** *Food Chem* 2012, **132**:1651-1659.
16. Pînzaru IA, Hădărugă DI, Hădărugă NG, Corpaş L, Peter F: **Hepatoprotective flavonoid bioconjugate/β-cyclodextrin nanoparticles: dsc-molecular modeling correlation.** *Digest Journal of Nanomaterials and Biostructures* 2011, **6**:1605-1617.
17. Casagrande R, Georgetti SR, Verri WAJ, Jabor JR, Santos AC, Fonseca MJV: **Evaluation of functional stability of quercetin as a raw material and in different topical formulations by its antilipoperoxidative activity.** *AAPS PharmSciTech* 2006, **7**:E1-E8.
18. Hădărugă DI, Hădărugă NG, Riviş A, Pârvu D: **Molecular modeling and docking studies on compositae biocompounds-cyclodextrin interactions.** *J Agroaliment Process Technol* 2009, **15**:273-282.
19. Hădărugă NG, Hădărugă DI, Păunescu V, Tatu C, Ordodi VL, Bandur G, Lupea AX: **Bioactive nanoparticles (6). Thermal stability of linoleic acid/α and β cyclodextrin complexes.** *Food Chem* 2006, **99**:500-508.
20. Hădărugă NG, Hădărugă DI, Lupea AX, Păunescu V, Tatu C: **Bioactive nanoparticles (7). Essential Oil from apiaceae and pinaceae family plants/β-cyclodextrin supramolecular systems.** *Revista de Chimie (Bucharest)* 2005, **56**:876-882.
21. Hădărugă DI, Hădărugă NG, Resiga D, Pode V, Dumbravă D, Lupea AX: **Obtaining and characterization of sage (*Salvia sclare* L.) Essential Oil/β-cyclodextrin supramolecular systems.** *Revista de Chimie (Bucharest)* 2007, **58**:566-573.
22. Hădărugă NG, Hădărugă DI, Riviş A, Păunescu V, Costescu C, Lupea AX: **Bioactive nanoparticles. Essential oil from lamiaceae family plants/β-cyclodextrin supramolecular systems.** *Revista de Chimie (Bucharest)* 2007, **58**:909-914.
23. Hădărugă DI, Hădărugă NG, Riviş A, Gruia A, Pînzaru IA: **Thermal and oxidative stability of the *Allium sativu* L. Bioactive compounds/α- and β-cyclodextrin nanoparticles.** *Revista de Chimie (Bucharest)* 2007, **58**:1009-1015.
24. Riviş A, Hădărugă NG, Hădărugă DI, Traşcă T, Drugă M, Pînzaru IA: **Bioactive nanoparticles. The complexation of odorant compounds with α- and β-cyclodextrin.** *Revista de Chimie (Bucharest)* 2008, **59**:149-153.
25. Costescu CI, Hădărugă NG, Hădărugă DI, Riviş A, Ardelean A, Lupea AX: **Bionanomaterials: synthesis, physico-chemical and multivariate analyses of the dicotyledonatae and pinatae essential oil/β-cyclodextrin nanoparticles.** *Revista de Chimie (Bucharest)* 2008, **59**:739-744.
26. Hădărugă DI, Hădărugă NG, Hermenean A, Riviş A, Păslaru V, Codina G: **Bionanomaterials: Thermal stability of the oleic acid/α- and β-cyclodextrin complexes.** *Revista de Chimie (Bucharest)* 2008, **59**:994-998.
27. Hădărugă DI, Hădărugă NG, Bandur G, Riviş A, Costescu C, Ordodi V, Ardelean A: ***Berberis vulgari* extract/β-cyclodextrin nanoparticles: synthesis and characterization.** *Revista de Chimie (Bucharest)* 2010, **61**:669-675.
28. Hădărugă DI, Hădărugă NG, Butnaru G, Tatu C, Gruia A: **Bioactive microparticles (10): thermal and oxidative stability of nicotine and its complex with β-cyclodextrin.** *J Incl Phenom Macrocycl Chem* 2010, **68**:155-164.
29. Hădărugă DI, Hădărugă NG, Merkh G, Isengard H-D: **Water content of fatty acid/cyclodextrin nanoparticles.** *J Agroaliment Process Technol* 2010, **16**:230-235.
30. Hădărugă NG, Hădărugă DI, Isengard H-D: **Water content of natural cyclodextrins and their essential oil complexes: a comparative study between Karl Fischer Titration and Thermal Methods.** *Food Chem* 2012, **132**:1741-1748.
31. Sapkal NP, Kilor VA, Bhusari KP, Daud AS: **Evaluation of some methods for preparing gliclazide-β-cyclodextrin Inclusion complexes.** *Trop J Pharm Res* 2007, **6**:833-840.
32. Patel HM, Suhagia BN, Shah SA, Rathod IS, Parmar VK: **Preparation and characterization of etoricoxib-β-cyclodextrin complexes prepared by the kneading method.** *Acta Pharm* 2007, **57**:351-359.

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