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Antioxidant activity evaluation of some *Matricaria chamomilla* L. extracts

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Abstract

The paper presents the obtaining, characterization, encapsulation and the antioxidant activity evaluation of some *Matricaria chamomilla* L. extracts. The *Matricaria chamomilla* L. essential oil has been obtained by steam distillation and steam distillation-extraction method. In order to evaluate the antioxidant capacity of chamomile, this was extracted with ethanolic solution, in different concentrations. The essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS), spectrophotometry and complexes with β-cyclodextrin by thermogravimetry (TG-DTG) and differential scanning calorimetry (DSC).

Keywords: β-cyclodextrin, nanoparticles, essential oil, thermogravimetry, gas chromatography-mass spectrometry, antioxidant activity

1. Introduction

Chamomile is a plant belonging to *Compositae* family – one of the oldest and precious medicinal species. The chamomile flowers and leaves contain an essential oil, whom main components, procamazulene, under high temperatures, are transforming in camazulene, compounds with healing properties. Also, the chamomile flowers contain mineral substances in proportion of 8-10%. The fresh obtained essential oil is blue colored with a bitterish taste and characteristic smell.

Antioxidants are substances which oppose oxidation or inhibit the reactions initiated by oxygen or peroxides, many of these substances being used in preserving different products (fats, foods and soaps). In general, natural products can serve as natural antioxidants source. Fenolic compounds, like vitamin E and flavonoids are typical antioxidants.

In case of chamomile essential oil, in order to preserve the volatile components and the active principles we are going to study the essential oil with β-cyclodextrin complexation.

2. Materials and method

Chamomile (*Matricaria chamomilla* L.) was purchased in different forms from many sources (dried – SC Plafar SA, Timişoara; fresh – in different periods of the year: April-June 2007). Ethanol 96% was obtained from Reactivul Bucureşti, the DPPH (1,1-diphenil-2-pycrilhydrazil) and the hexane were purchased from Merck&Co., Inc., New Jersey. The solvents were obtained from FlukaChemie AG.

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Obtaining the chamomile essential oil through steam distillation. 25 g vegetal material (fresh, partial dried or dried chamomile flowers) were broken up and placed in a 500 ml balloon filled with 250 ml water. It is then attached a distillation system and also a dropping funnel to the balloon. The mixture is warmed up to boiling point and the organic layer is gathered in a separating funnel from which the blue essential oil is gathered. The raw essential oil can be separated and processed or it may be extracted for 2 more times in 50 ml dichloromethane in an extraction-separation funnel. The extracts are then being dried over anhydrous Na_2SO_4, filtered, and the solvent is being removed with a rotary evaporator without heating. The obtained essential oil is measured having it's raw efficiency 0,07%.

Obtaining the chamomile essential oil through steam distillation-extraction (SDE). 20 g vegetal material and 100 ml water are being put in a 250 ml balloon while 8 ml extracting solvent (hexane) is being put in a pear like balloon. The balloons are then connected to the steam distillation-extraction system (fig. 1). The balloon containing the vegetal material is heated up to 69-70ºC on a water bath. The distillation-extraction process is completed in about 1-3 hours, followed by the cooling of the system and the extract collection from the 25 ml balloon. This extract dries over anhydrous Na_2SO_4, is filtered and the organic solvent is slowly distilled under vacuum. The obtained essential oil is measured and it's efficiency and physical-chemical features are being determined.

The essential oil extraction conditions and the results obtained after this process are presented in table 1a and 1b.

Nanoencapsulation of the chamomile essential oil in cyclodextrins. The β-cyclodextrin quantity indicated in table 3 is measured and dissolved in 2 ml distilled water at 50ºC. Then an ethanolic solution of the chamomile essential oil (the weight corresponding to essential oil main compounds (chamazulene and oxide-bisabolol) : βCD molar ratio of 1 : 1) were slowly added under continuous stirring. The solution was then stirred another 30 minutes and slowly cooled, on a water bath, in about 4 h. The complex was filtered, washed with ethanol and dried in exicator. The obtained results are shown in table 2.

GC-MS analysis of the chamomile essential oil. For the analysis of the chamomile essential oil a Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass spectroscopy detector (GC-MS) system was used. A HP-5 MS capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) was used for the GC system. The temperature program was set up from 50°C to 250°C with 6°C/ min, both the injector and detector temperatures were 250ºC and He was used as carrier gas. The injection volume was 2 μl. Ionization energy EI of 70eV was used for mass spectroscopy detector, with a source temperature of 150°C, scan range 50-300 amu, scan rate $1s^{-1}$. Compounds separated by GC were identified by matching the experimental mass spectra with those from the NIST/EPA/NIH Mass Spectral Library 2.0.

Obtaining Matricaria chamomilla L. flavonoid extracts. In a 100 ml balloon, the appropriate quantity of vegetal material, as shown in table 3, is introduced, as well as 20 ml solution of ethanol-water, having the indicated concentration. The mixture is cold stirred for 1 h, is filtered and washed in 4 ml ethanolic solution. The extraction for the 30 minutes ethanol reflux is being done in the same way. Then, the samples are cooled down, filtered and washed in 4 ml ethanolic solution with the same concentration. The extracts obtained in the end are evaluated for their antioxidant activity.

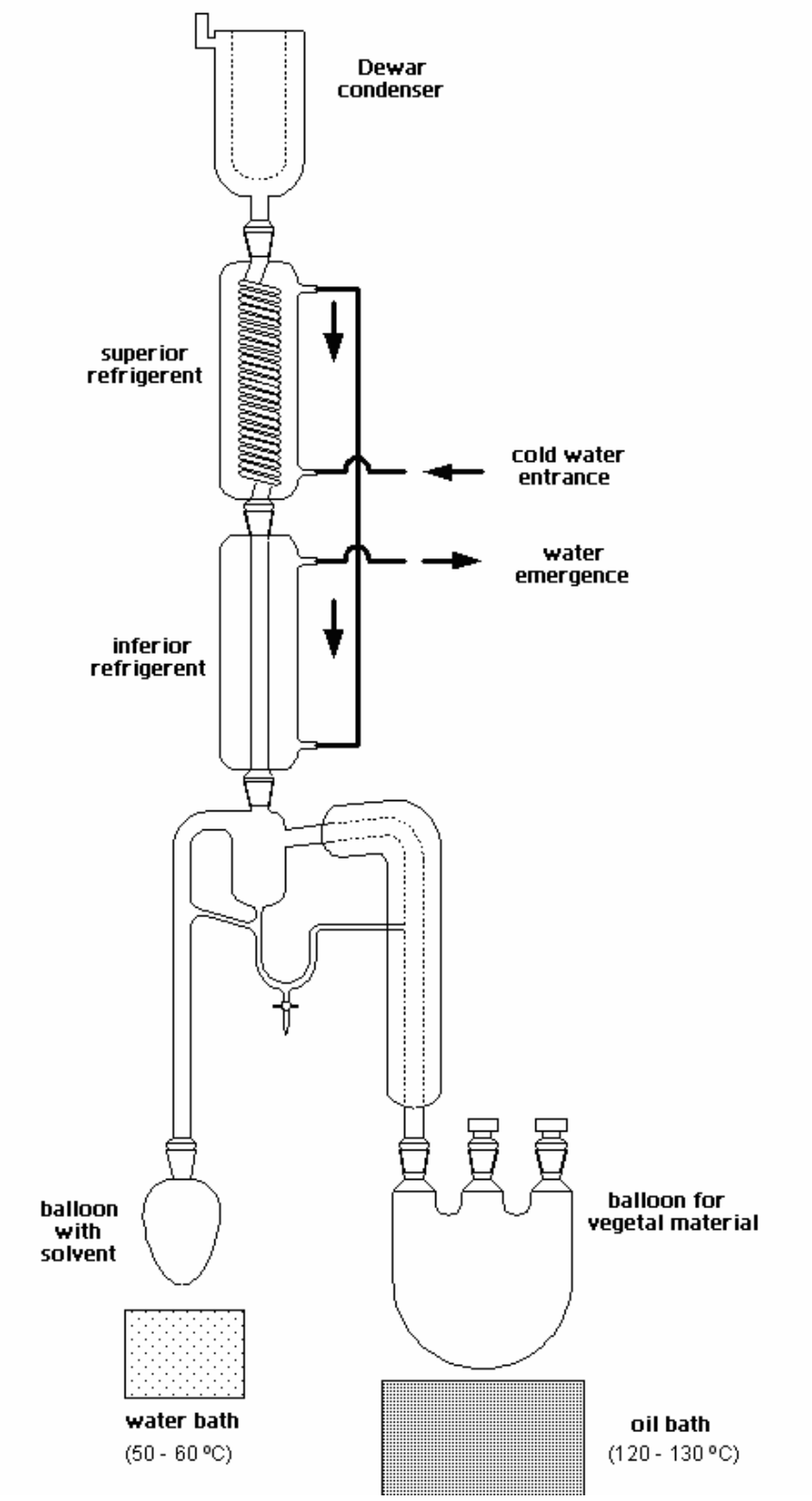

Fig. 1. Steam distillation-extraction system (SDE)

Table 3. Extraction conditions for obtaining the flavonoid extracts with antioxidant activity

No.	Code	m (sample) (g)	V (solvent) (mL)	V (washing) (mL)	Description
1	C9	2	20	4	Chamomile flowers extract obtained through cold extraction with ethanol 96%.
2	C9A	Chamomile flowers extract obtained through cold extraction with ethanol 96% diluted with 2.5 mL ethanol 96%.			
3	C9B	Chamomile flowers extract obtained through cold extraction code [C9A] diluted with 2.5 mL ethanol 96%.			
4	C9C	Chamomile flowers extract obtained through cold extraction code [C9B] diluted with 2.5 mL ethanol 96%.			
5	C10	2	20	4	Chamomile flowers extract obtained through cold extraction with ethanol 60%.
6	C11	2	20	4	Chamomile flowers extract obtained through cold extraction with ethanol 20%.
7	C11A	Chamomile extract obtained through cold extraction code [C11] upon which we added 2.5 mL ethanol 96%			
8	C12	2	20	4	Chamomile flowers extract obtained through cold extraction with ethanol 0% (distilled water)
9	C12A	Chamomile extract obtained through cold extraction code [C12] upon which we added 2.5 mL ethanol 96%			
10	1	2.5	20	4	Chamomile root extract obtained through cold extraction with ethanol 96%.
11	6	4	20	4	Chamomile stem extract obtained through cold extraction with ethanol 60%.
12	10	2.5	20	4	Chamomile leaves extract obtained through cold extraction with ethanol 60%.
13	12	2.5	20	4	Chamomile leaves extract obtained through cold extraction with ethanol 0% (distilled water)
14	13	2	20	4	Chamomile flowers extract obtained through warm extraction with ethanol 96%.
15	13a	Chamomile flowers extract obtained through warm extraction with ethanol 96% upon which we added 2.5 mL ethanol 96%			
16	13b	Chamomile extract obtained through warm extraction code [13a] upon which we added 2.5 mL ethanol 96%			
17	13c	Chamomile extract obtained through warm extraction code [13b] upon which we added 2.5 mL ethanol 96%			
18	13d	Chamomile extract obtained through warm extraction code [13c] upon which we added 2.5 mL ethanol 96%			
19	15	2	20	4	Chamomile flowers extract obtained through warm extraction with ethanol 20%.
20	16	2	20	4	Chamomile flowers extract obtained through warm extraction with ethanol 0% (distilled water)
21	16a	Chamomile flowers extract obtained through warm extraction with distilled water upon which we added 2.5 mL ethanol 96%			
22	16b	Chamomile flowers extract obtained through warm extraction with distilled water code [16a] upon which we added 2.5 mL ethanol 96%			

Spectrofotometric UV-VIS analysis and antioxidant activity determination. The extracts obtained through cool extraction and refluxes were analyzed as concerns their antioxidant activity using a Lambda EZ Series Perkin Elmer UV-VIS spectrophotometer. The data obtained were processed using PESSW program (version 1.2, Revision E). In the sample container were introduced 2,4 ml EtOH 96% and 0,3 ml of extract, then 0,3 ml DPPH 1mM and the recording began. The reaction mixture contains ethanol, 1 mM DPPH and testing samples. After stirring, absorbance at 517 nm was recorded. In time, the solution looses color, while the DPPH reacts with free radicals from the system, and the reaction progress is spectrophotometricaly monitorised. All notes were made having the EtOH with the appropriate concentration as reference. Antioxidant activity of the extracts was determined for primary, as well as for diluted extracts (table 3).

TG-DTG analysis. The thermogravimetric analysis for the essential oil/βCD complexes was performed on a Netzsch TG-209 apparatus, using a temperature program of 20-550ºC, with a heating rate of 4ºC/min. All analysis was conducted under nitrogen atmosphere.

DSC analysis. The differential scanning calorimetric analysis for the chamomile essential oil/βCD complex was performed on a DSC Netzsch-204 apparatus, using a temperature program of -50÷450ºC, with a heating rate of 4ºC/min, the cooling being realized with liquid nitrogen. The data acquisition was realized using a specific DSC Netzsch 204-Acquisition Soft/2000 and the data processing was realized with Netzsch Proteus-Thermal Analysis (vesion 4.0/2000).

3. Results and discussion

For the chamomile essential oil separation, using steam distillation, were obtained relative low yield (appreciatively 0,07%), reported to dry vegetal material. In case o fresh vegetal material the yields were even lower, probable because the plants were very young and they presented a very low contents of essential oil.

The GC-Ms analysis shown that 88 compounds, from different classes, were separated, the most concentrated ones being sesquiterpenes (fig. 2).

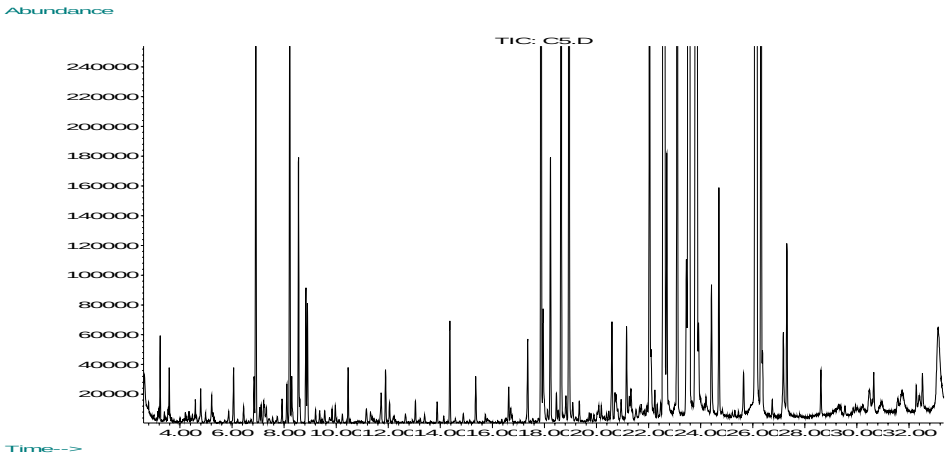

Fig. 2. Gas chromatogram from the GC-MS analysis of the chamomile essential oil

The main sesquiterpenes identified in the chamomile essential oil were camazulene (19,9%), α-bisabolol (20,9%), A and B bisabolol-oxides (21,6% and respectively 1,2%) and β-farnesen (3,1%). In lower concentrations were identified α- and β-caryophyllene, caryophyllene-oxide and spathulenol, and also some monoterpenes like β-phellandrene (0,8%), limonene (0,8%), β-ocymene (0,4%) and γ-terpinen (0,2%) (table 4).

Table 4. The main compounds identified in chamomile essential oil

No.	RT (min)	Conc. (%)	Compound name (MS identification)
1	6.057	0.1	α-Pinene
2	6.915	0.7	β-Phellandrene
3	8.108	0.1	m-Cymene
4	8.213	0.8	Limonene
5	8.29	0.1	α-cis-Ocimene
6	8.554	0.4	β-cis-Ocimen
7	8.836	0.2	Artemisia ketone
8	8.895	0.2	γ-Terpinen
9	11.727	0.1	Borneol
10	11.897	0.1	3-Cyclohexen-1-ol
11	17.873	3.1	β-Farnesen
12	18.231	0.5	Caryophyllene oxide
13	18.942	1.7	o-Menth-8-ene, 4-isopropylidene-1-vinyl-
14	20.593	0.2	(-)-Spathulenol
15	22.033	1.2	α-Bisabolol oxide B
16	**22.608**	**20.9**	**α-Bisabolol**
17	22.702	0.4	Caryophyllene oxide
18	23.096	1.6	cis-Geranylacetone
19	23.449	0.4	Coumarin, 7-methoxy
20	**23.566**	**19.9**	**Camazulene**
21	**23.854**	**21.6**	**Bisabolol oxide A**
22	24.7	0.4	cis-Z-α-Bisabolene epoxide
23	26.151	19.4	1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiyn-1-ylidene)- ?
24	27.309	0.3	7-Isopropyl-1,4-dimethyl-2-azulenol
		5.6	*Alţi compuşi*

For the complexation of chamomile essential oil in β-cyclodextrin was obtained a yield of almost 60%, the TG-DTG and DSC analysis showing clearly the molding of the complex.

Matricaria chamomilla L. extraction and the antioxidant activity determination. In order to determine the antioxidant activity of chamomile, it was necessary to extract with ethanolic solution different concentrations of flavonoids. The antioxidant activity was determined using DPPH method. The reaction speeds assessment of this compound, in presence of the studied samples, needed to obtain a standard curve *Absorbance (517 nm) = f (concentration, mM)* (fig. 3).

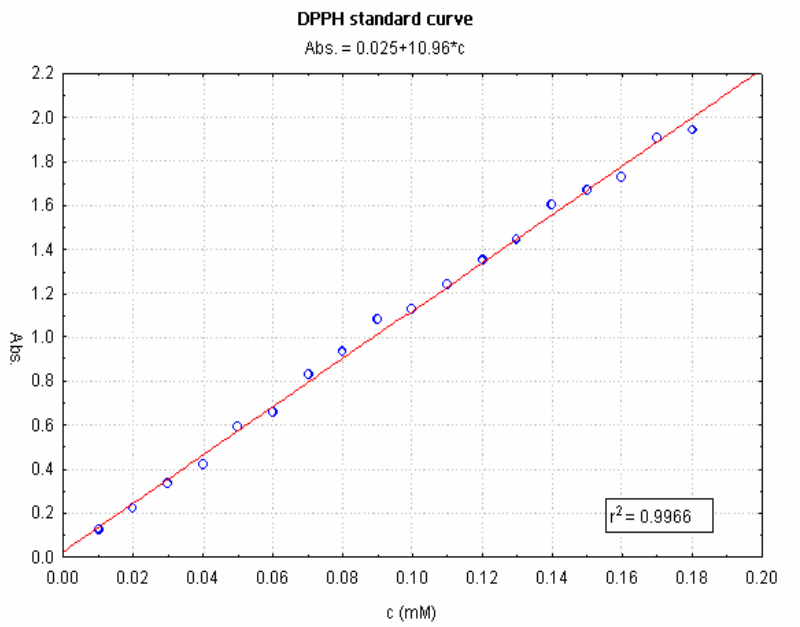

Fig. 3. UV-VIS spectra for DPPH standard solutions and standard curve *Abs = f (c, mM)*

Observing, in time, the variation of DPPH solution absorbance in presence of *Matricaria chamomilla* L. ethanol-water extracts sample (flowers) (for undiluted and gradual diluted samples) through spectrophotometric method, indicated, in the majority of the cases, a conversely logarithmic variation (fig. 4 and fig. 5).

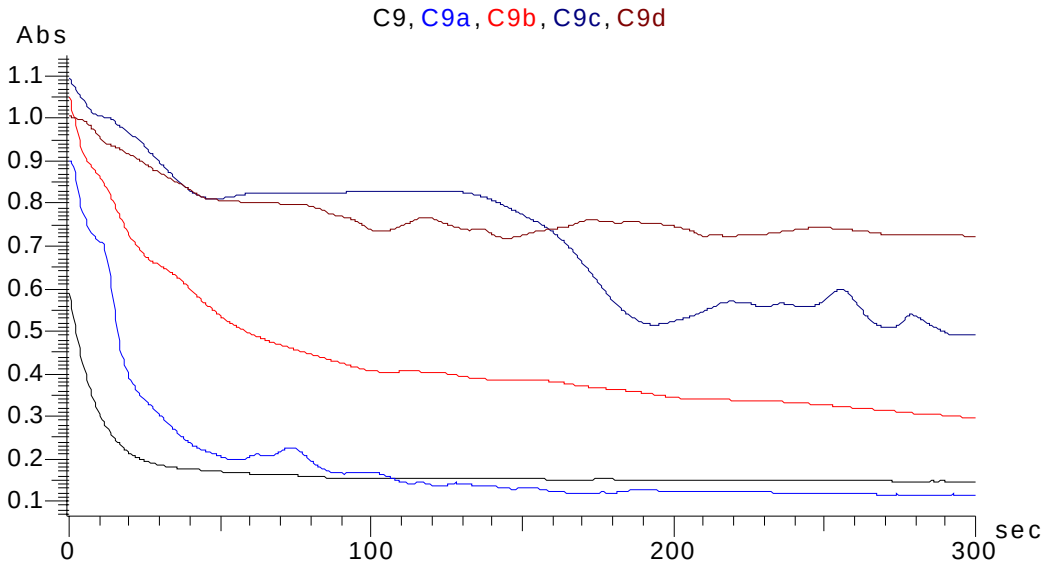

Fig. 4. Superposition of the *Abs = f (time, s)* curves for *C9* undiluted and diluted samples (a-d)

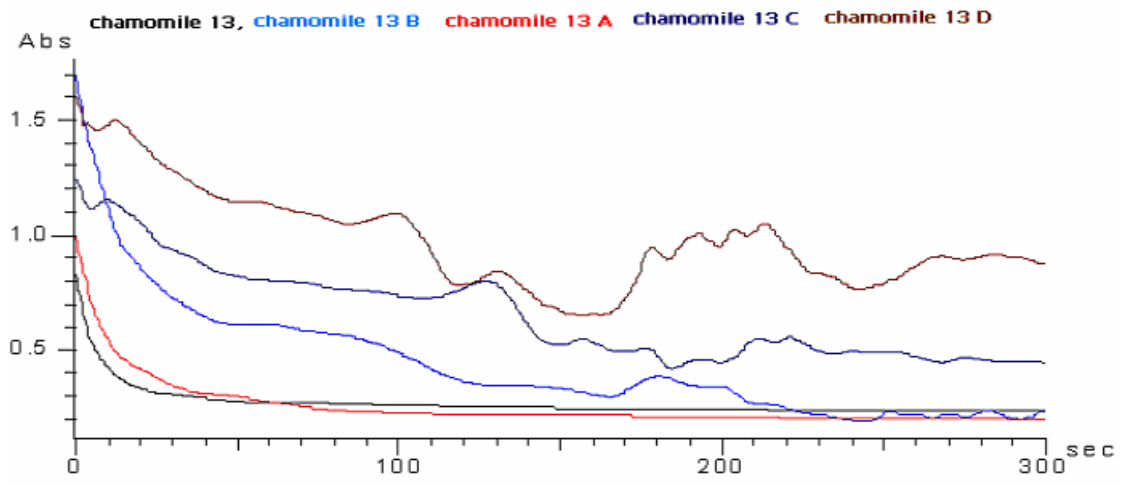

Fig. 5. Superposition of the *Abs = f (time, s)* curves for *C13* undiluted and diluted samples (a-d)

In order to evaluate the antioxidant activity based on these determinations, it has been obtained the dependence curves of relative absorbance (*A%*) as a report between absorbance at *t* time and initial absorbance (t = 0):

$$A\%(t) = \frac{A_{517nm}(t)}{A_{517nm}(t=0)} \cdot 100$$

Meaningful results were obtained just in case of chamomile flowers extracts obtained through cold and warm extraction. In case of stems and roots the absorbance variation wasn't important.

In case of samples obtained through cold extraction the antioxidant activity was important, *A%* values being almost 10% for all undiluted samples (fig. 6-9).

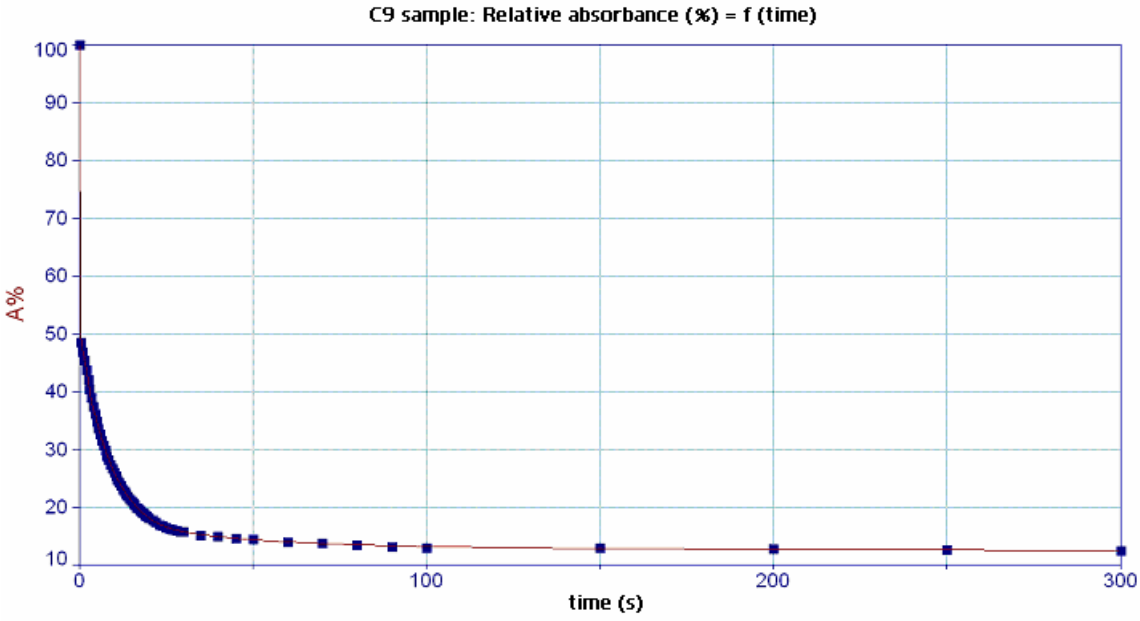

Fig. 6. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through cold extraction in ethanol 96% (undiluted sample)

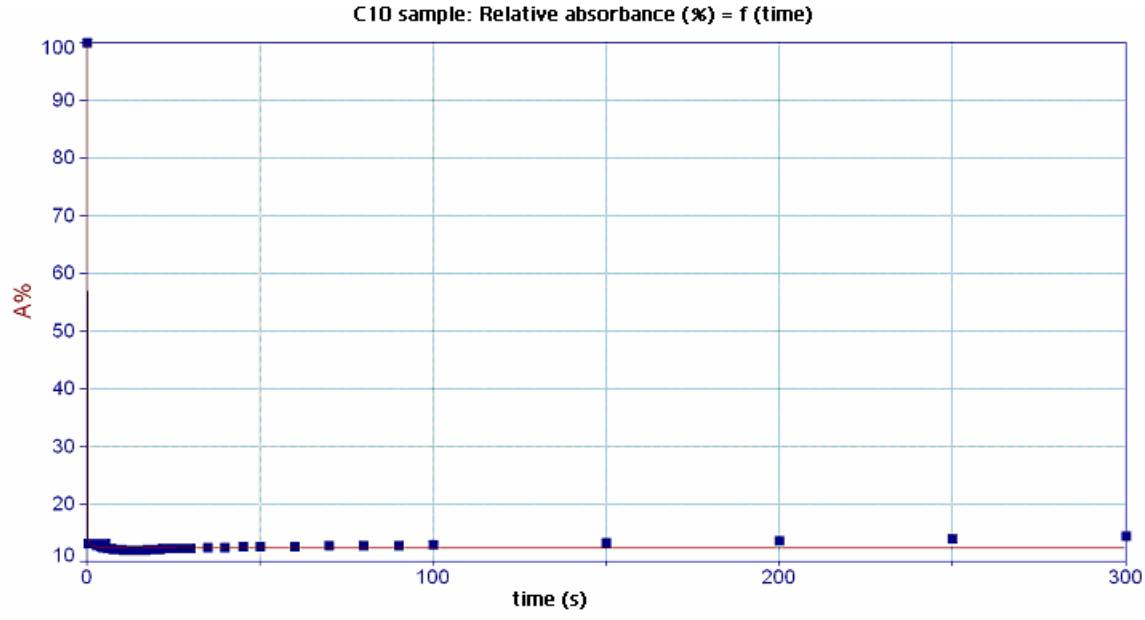

Fig. 7. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through cold extraction in ethanol 60% (undiluted sample)

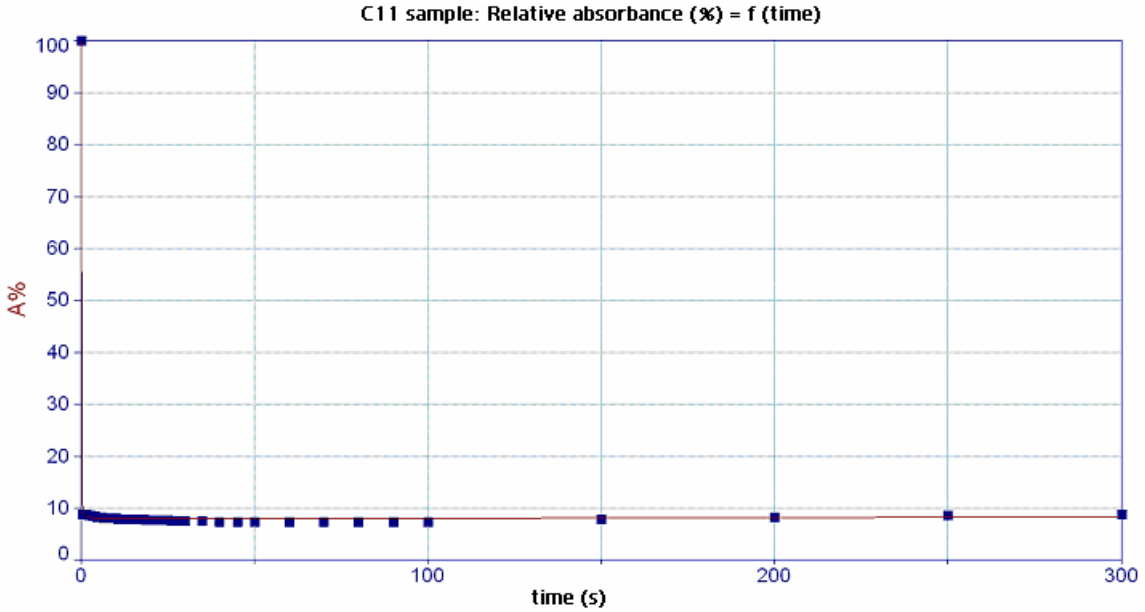

Fig. 8. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through cold extraction in ethanol 20% (undiluted sample)

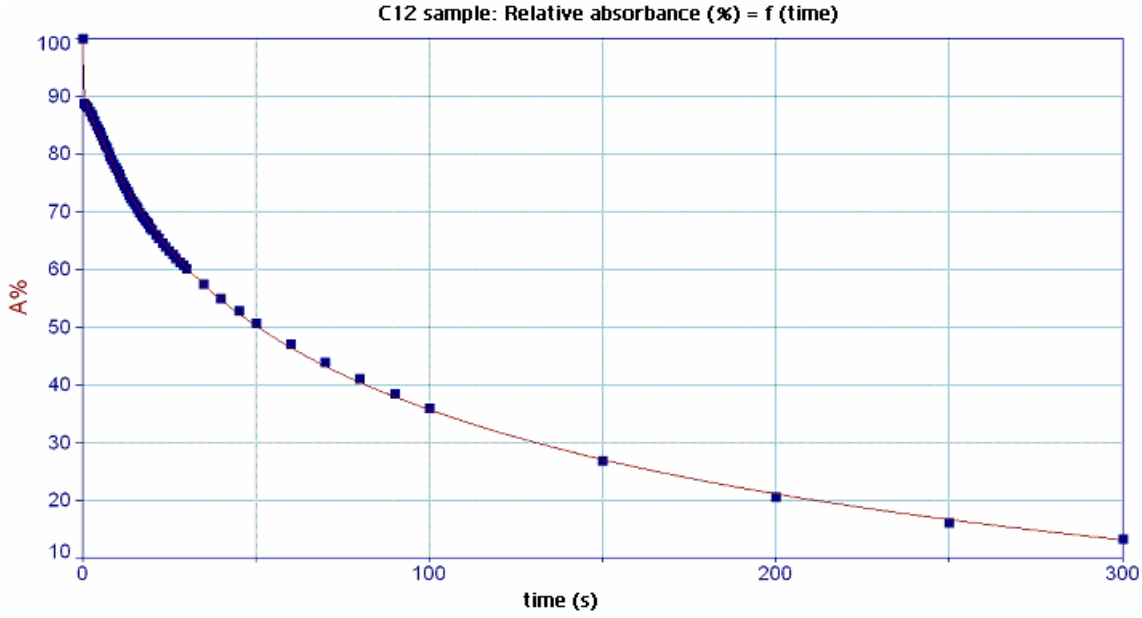

Fig. 9. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through cold extraction in water (undiluted sample)

On the basis of these DPPH solutions absorbance variations in time, in the presence of the extract samples, and respectively on the basis of the DPPH standard curve *Abs (517 nm) = f (conc., mM)*, we could determine the variation of DPPH concentration in time. Also, we could evaluate the average reaction speeds of DPPH on the pseudo linear section of the curve concentration (μM) = f (time, s), in concordance with the following relation:

$$v = -\frac{dc_{DPPH}}{dt} \quad (\mu M/s)$$

In the cases presented above we determine the average reaction speeds, which were situated between 0,2 and 9 μM, for the samples obtained through cold extraction. The diluted samples presented lower speeds comparatively with undiluted ones (table 5 and fig. 10). Samples extracted with medium concentrated ethanol (60% and 20%) presented the highest reaction speeds.

Table 5. Average reaction speeds of DPPH for *Matricaria chamomilla* L. samples obtained through cold extraction

No.	Code	Description	$v_{average}$ (μM/s)
1	C9	*M.chamomilla* extract in EtOH96%, undiluted	2.0
2	C9a	*M.chamomilla* extract in EtOH96%, *a* dilution	2.1
3	C10	*M.chamomilla* extract in EtOH60%, undiluted	8.6
4	C11	*M.chamomilla* extract in EtOH20%, undiluted	9.0
5	C11a	*M.chamomilla* extract in EtOH20%, *a* dilution	7.2
6	C12	*M.chamomilla* extract in water, undiluted	1.3
7	C12a	*M.chamomilla* extract in water, *a* dilution	0.2

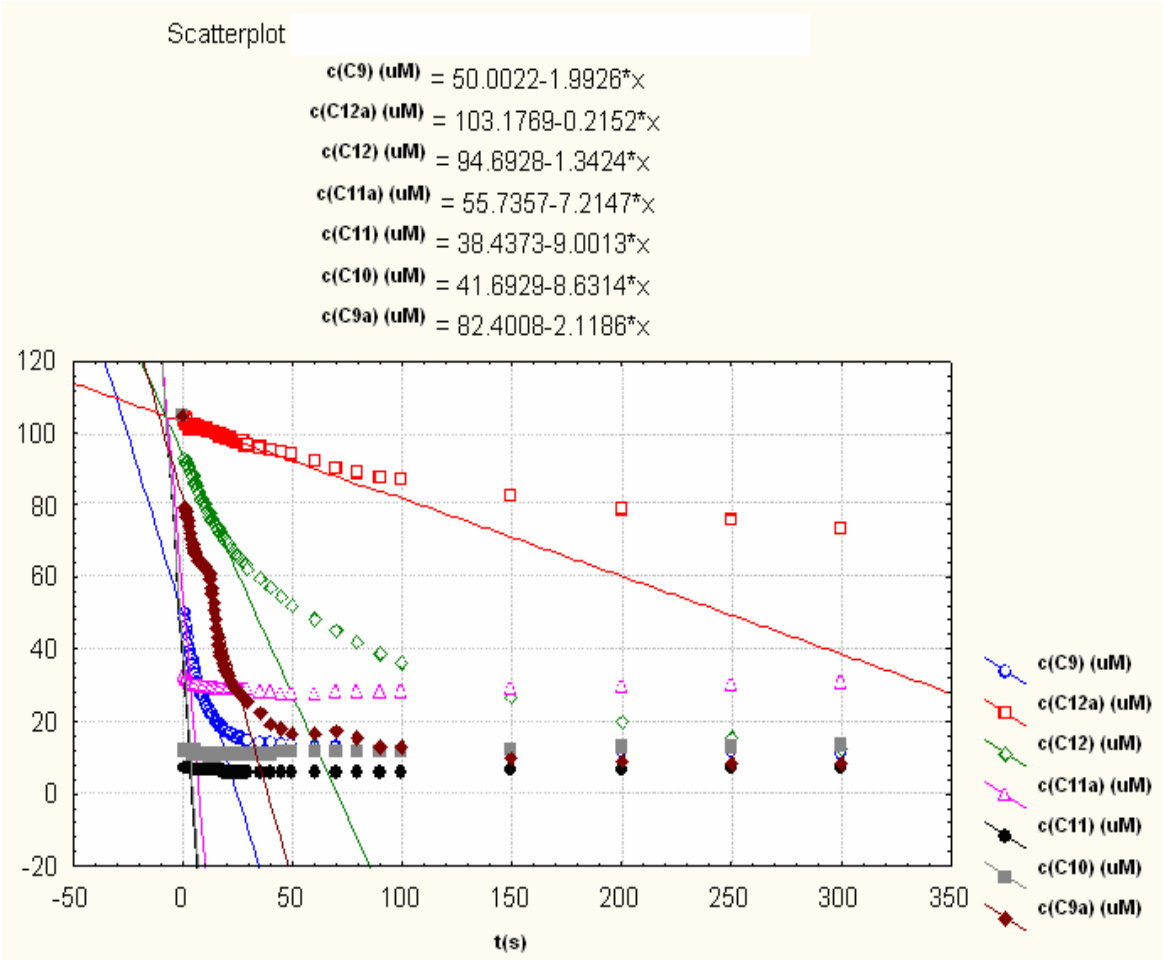

Fig. 10. DPPH concentration (μM) dependence on reaction time, in presence of undiluted and diluted *Matricaria chamomilla* L. samples, obtained through cold extraction

In the case of samples obtained through warm extraction, the antioxidant activity (relative absorbance) *A%* was appreciatively 20%, in the case of extracts with ethanol 96%, for undiluted sample and also for gradual diluted samples (fig. 11-13). The average reaction speeds on the pseudo linear section of the curve are decreasing with the dilution increasing, being situated between 8,5 and 0,7 μM/s (fig. 14).

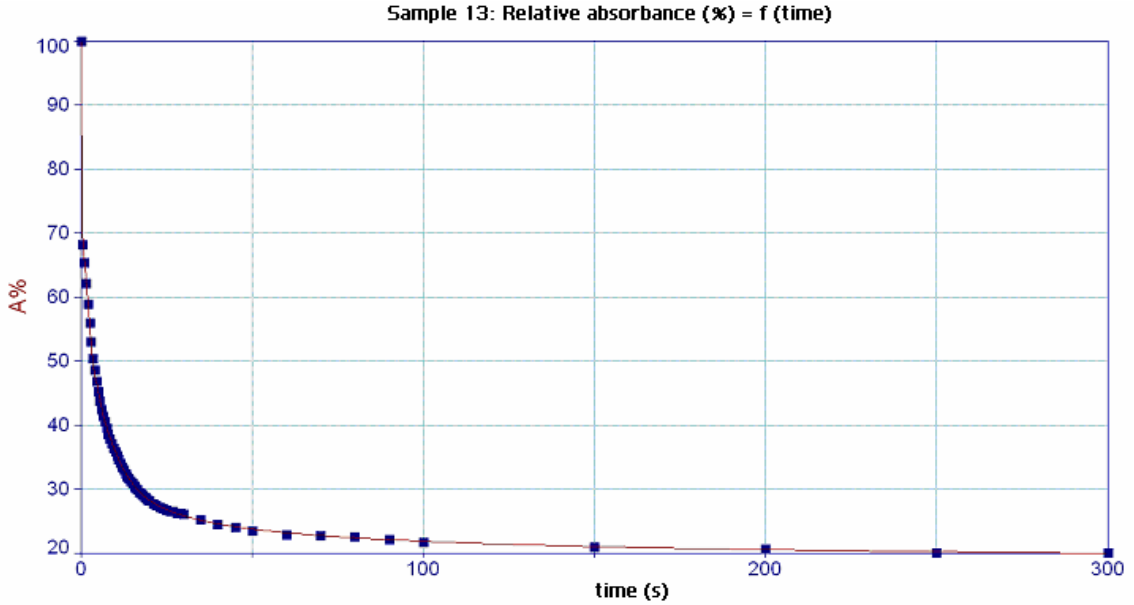

Fig. 11. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through warm extraction in ethanol 96%

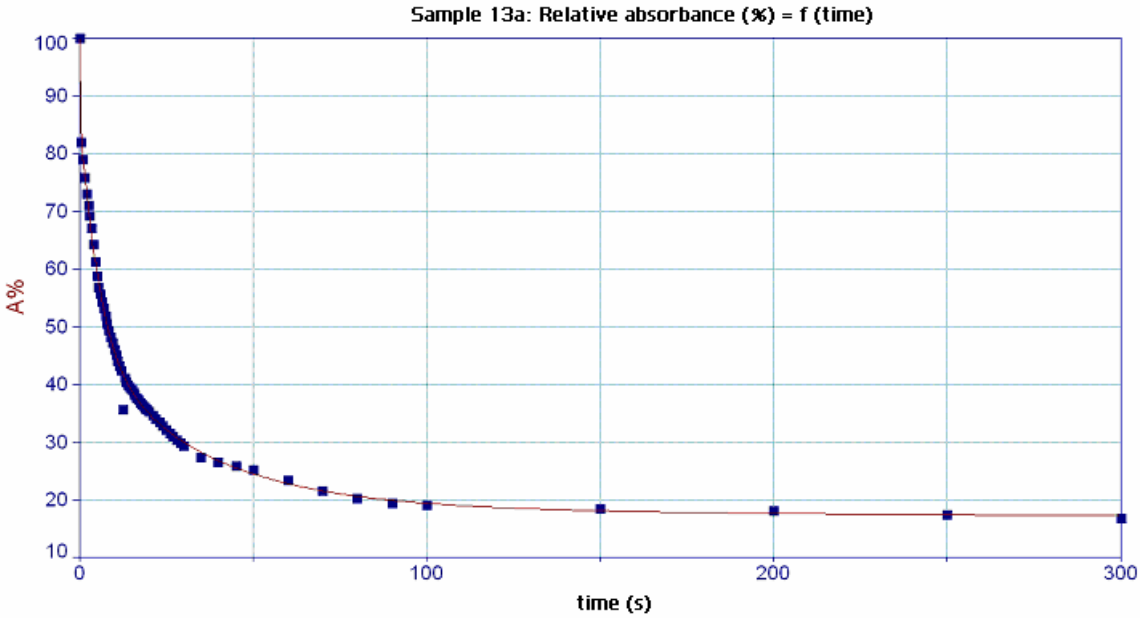

Fig. 12. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through warm extraction in ethanol 96% (*a* dilution)

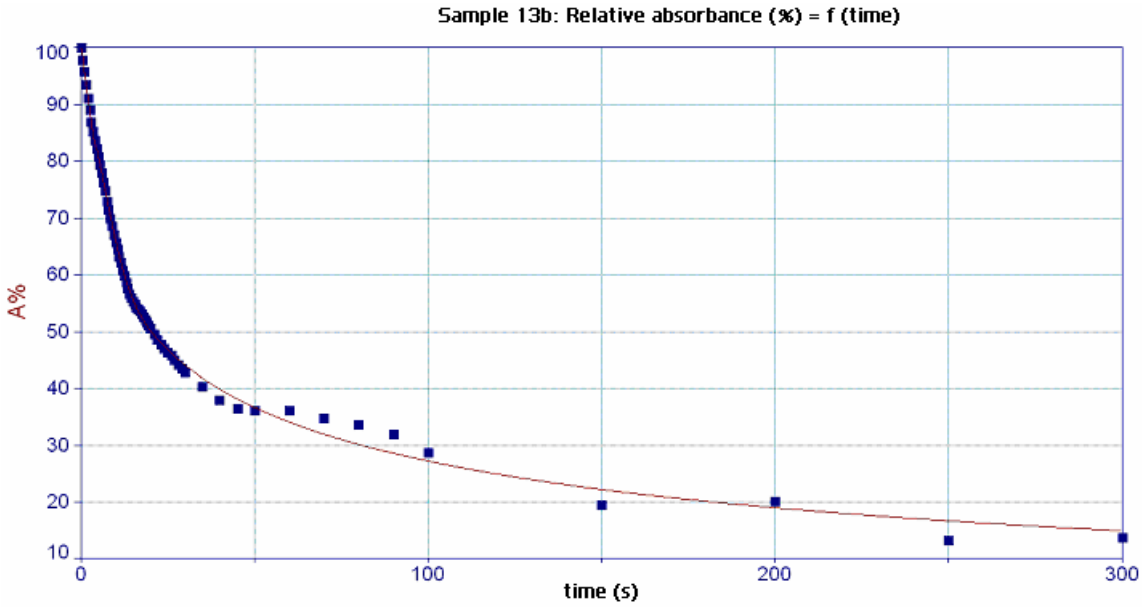

Fig. 13. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through warm extraction in ethanol 96% (*b* dilution)

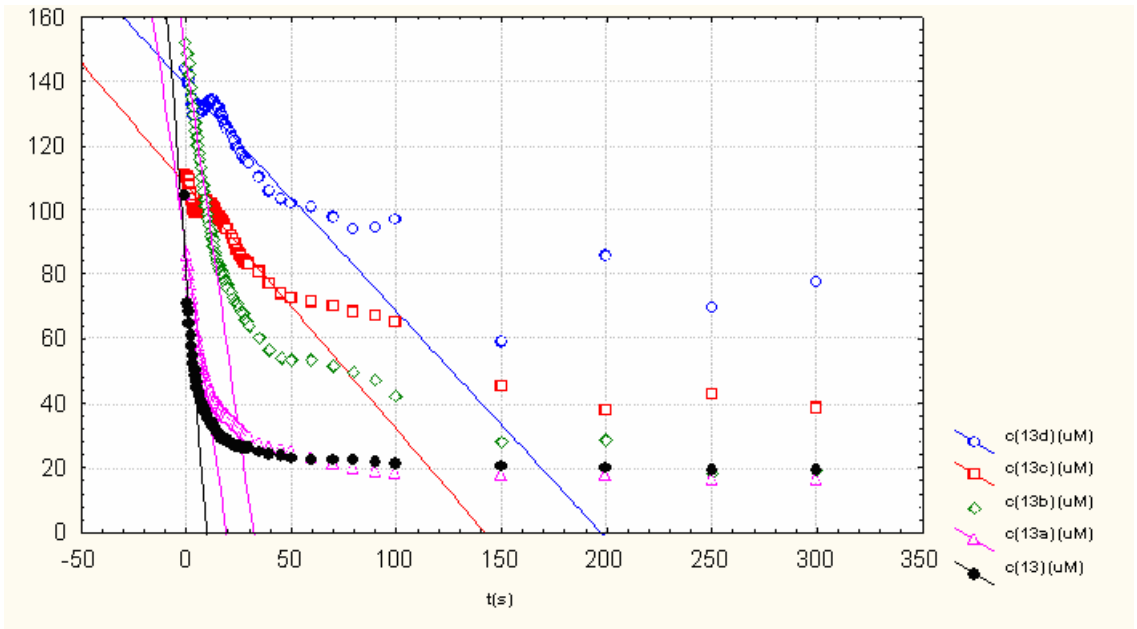

Fig. 14. DPPH concentration (µM) dependence on reaction time, in presence of undiluted and diluted *Matricaria chamomilla* L. samples, obtained through cold extraction with EtOH 96%

Lower antioxidant activity was observed in the case of samples obtained through warm extraction with diluted ethanol or water, *A%* values being situated between 30 and 55% (fig. 15-16). Reaction speeds varies similarly, being situated between 5,8 and 1,4 µM/s and for undiluted samples these values weren't altering much (table 6 and fig. 17).

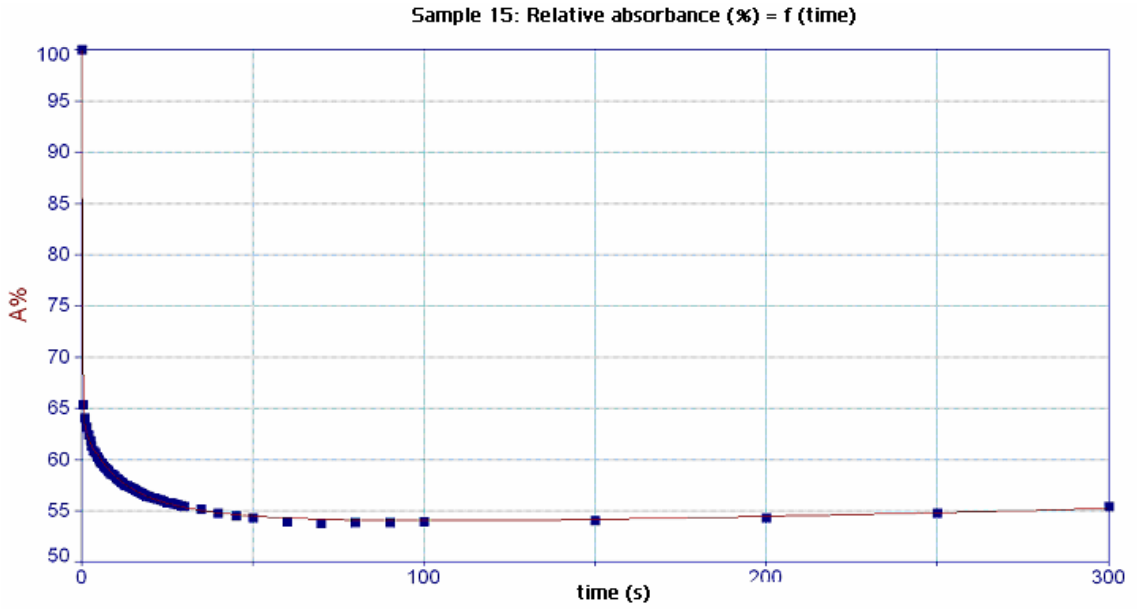

Fig. 15. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through warm extraction in ethanol 20%

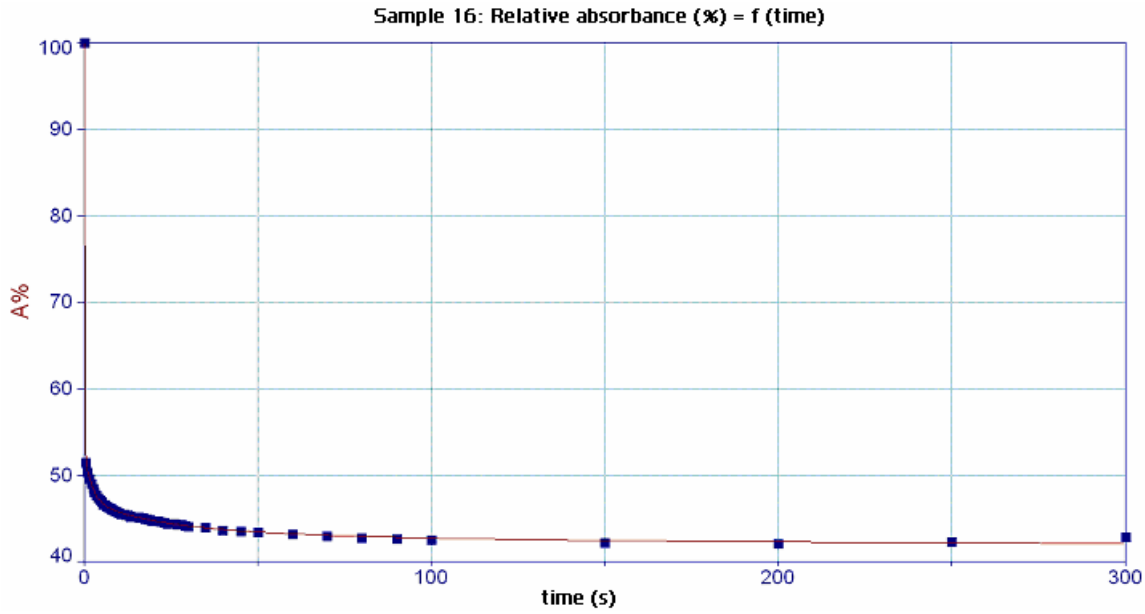

Fig. 16. Antioxidant activity of *Matricaria chamomilla* L. extract obtained through warm extraction in water

Table 6. Average reaction speeds of DPPH for *Matricaria chamomilla* L. samples obtained through warm extraction with diluted ethanol or with water

No.	Code	Description	$v_{average}$ (µM/s)
1	15	*M.chamomilla* extract obtained through warm extraction in EtOH20%, undiluted	4.6
2	16	*M.chamomilla* extract obtained through warm extraction in water, undiluted	5.8
3	16a	*M.chamomilla* extract obtained through warm extraction in water, *a* dilution	4.0
4	16b	*M.chamomilla* extract obtained through warm extraction in water, *b* dilution	1.4

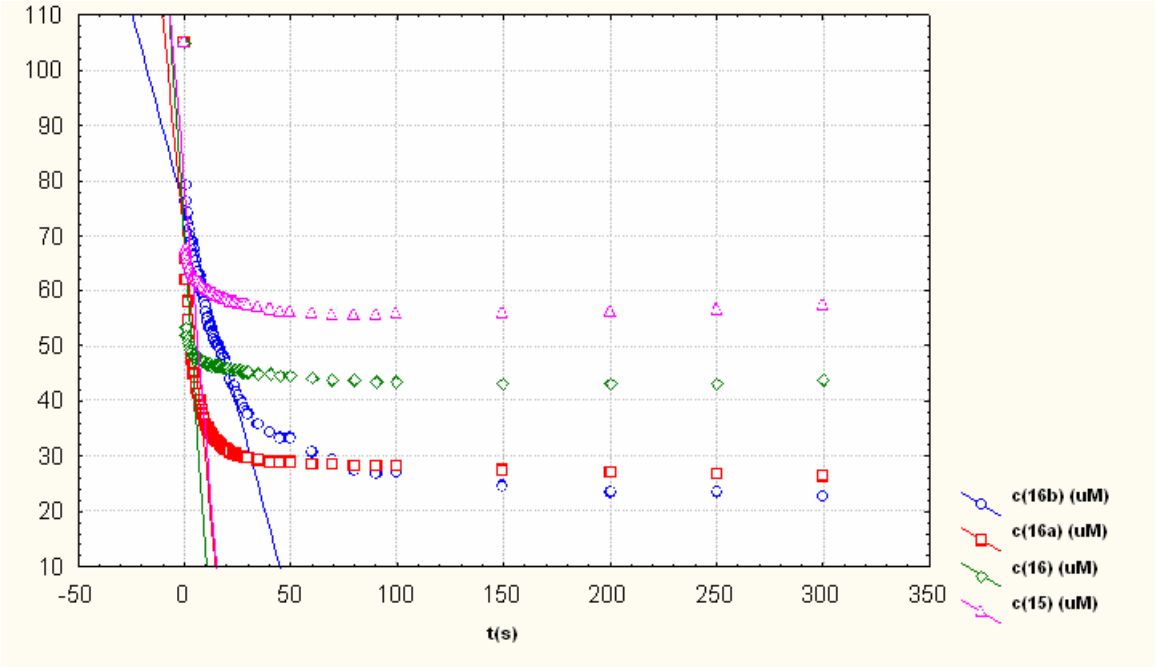

Fig. 17. DPPH concentration (µM) dependence on reaction time, in presence of undiluted and diluted *Matricaria chamomilla* L. samples, obtained through warm extraction with EtOH 20% and with water

4. Conclusion

From the performed studies which concern the obtaining, analysis, nanoencapsulation and the evaluation of antioxidant properties of *Matricaria chamomilla* L., it can be indicated the following conclusions:

- the essential oil extraction process from dried chamomile flowers was performed with good yield (0,07%), but through steam distillation-extraction process from fresh vegetal material we couldn't separate the essential oil with efficient yield;

- from the GC-MS analysis, the main compounds identified in the essential oil were especially sesquiterpenes, but also some monoterpenes. The highest concentrations were calculated for chamazulene, α-bisabolol and A bisabolol-oxide (appreciatively the same concentration 20-21% for each component);

- chamomile essential oil complexation with β-cyclodextrin was realized with a yield of 60%, the TG and DSC data indicating the complex formation;

- all the chamomile flowers extract samples obtained through warm or cold extraction

with ethanol-water solutions presented an important antioxidant activity, while the analyzed samples from stem, root or leaves presented a very poor antioxidant activity;

- the average reaction speeds on the pseudo linear section of the curves c = f (t) were situated in the range of 0,2-9 μM/s, higher speeds being observed for the ethanolic (60% and 20%) extracts (both for the samples obtained through warm or cold extraction).

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