Development of hydrosoluble gels with *Crataegus monogyna* extracts for topical application: evaluation of antioxidant activity of the final formulations

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ABSTRACT

Crataegus monogyna, commonly known as hawthorn, is notorious for having different ethnopharmacological applications. The antioxidant activity of methanolic extracts from its flowers and fruits in several stages of development was formerly reported by us. Therefore it is believable that the extracts of its botanical parts might have great potential to be incorporated in innovative pharmaceutical formulations. Accordingly, several paraben free, carbopol 940 based semisolid formulations were prepared with different hawthorn parts extracts. The antioxidant properties of ethanolic and aqueous hawthorn extracts, as well as their corresponding hydrosoluble gels, were screened. Ethanolic extracts proved to be more effective, while flowers and unripe fruits were the most bioactive hawthorn parts. The prepared hydrosoluble gels, that presented an adequate consistency, a pleasant colour and a non greasy texture, kept almost entirely the antioxidant activity exhibited by the extracts from which they were prepared. In addition, the hydrosoluble gels were tested and promptly absorbed by the skin and did not show significant pH alterations during the 90 days of observation. Despite the need of further tests (viscosity, extrudability, spreadability or transepidermal water loss), the prepared formulations demonstrate interesting attributes to be explored as potential dermopharmaceutical products.

***Keywords**:* hydrosoluble gels, hawthorn; ethnopharmacology; antioxidant activity; bioactive compounds.

1. Introduction

Historically, medicinal preparations are derived from plants, whether in the simple form of plant parts, or as crude extracts or mixtures, among others. Today a substantial number of drugs are developed from plants which are active against a number of diseases (Haneefa et al., 2010). This might be due to the fact that plants are a particularly good source of compounds with antioxidant properties. Different wild plants from Northeastern Portugal, belonging to some botanical families with several ethnomedicinal uses such as Rosaceae (*Crataegus monogyna*, *Prunus spinosa*, *Rosa canina*) (Barros et al., 2010b, 2011), Lamiaceae (*Glechoma hederaceae*, *Mentha* sp.pl., *Origanum vulgare*, *Thymus mastichina*) (Barros et al., 2010a; Guimarães et al., 2011), Apiaceae (*Foeniculum vulgare*) (Guimarães et al., 2011), Ericaceae (*Arbutus unedo*) (Barros et al., 2010b), Malvaceae (*Malva sylvestris*) (Barros et al., 2010c) and Verbenaceae (*Aloysia citrodora*) (Guimarães et al., 2011) were previously evaluated in alcoholic or aqueous extracts, and showed high antioxidant potential, especially in the case of *Crataegus monogyna* Jacq. unripe fruits (EC_{50} values under 25 μg/ml of methanolic extract; Barros et al., 2011). Total antioxidant activity of fruits and vegetables is thought to reflect concentrations of various antioxidant groups, the most commonly reported of which are phenolic compounds, ascorbic acid, α-tocopherol, β-carotene and glutathione (Pennington and Fisher, 2009). *C. monogyna*, commonly known as hawthorn, revealed considerable concentrations of phenolics, flavonoids, ascorbic acid, tocopherols, β-carotene, saturated (hexadecanoic and tricosanoic) and polyunsaturated ((9*Z*,12*Z*)-octadeca-9,12-dienoic and (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic) fatty acids (Barros et al., 2011). Hawthorn is indeed one of the species that is highly recommended in folk medicine, being regarded as particularly important in the management and prevention of age-related diseases (for instance, cardiovascular disease, atherosclerosis, arthritis, and hypertension), nervous system disorders (such as migraines, confusion, irritability and memory loss) and treatment of upper respiratory

infections, cellulite, obesity and menopause disturbances (Camejo-Rodrigues et al., 2003; Novais et al., 2004; Neves et al., 2009; Carvalho, 2010;). Furthermore, the juice of its fruits is a topical preparation for skin application that relieves pain and stiffness (Carvalho, 2010). The skin is an important protective barrier between the environment and the inner milieu, being highly exposed to oxidative stress, either from exogenous as well as endogenous sources (Masaki, 2010; Portugal et al., 2007). Exogenous sources include environmental factors such as air pollutants, ionizing and non-ionizing radiation (Kohen and Nyska, 2002), while endogenous sources consist of reactive species formed from activated phagocytes and enzymes which produce active oxygen metabolites (Rieger and Pains, 1993). However, oral and dermal administered natural antioxidants such as superoxide dismutase (SOD), ferulic acid and flavonoids can protect the skin against the oxidative activity of UV irradiation (Graf, 1992; Montenegro et al., 1995). In fact, there is evidence that topical applications of compounds with free radical scavenging properties in patients do protect tissues from oxidative damage (Meenakshi et al., 2006) and the employment of medicinal plants in dermatological and cosmetic products is increasing. The collateral effects originated from their use are much smaller if compared with the effects caused by synthetic products. Nevertheless, plant formulations must be standardized and studied in order to guarantee their quality, safety and efficacy. Research aimed at identifying, characterizing and relating pharmacological and toxic effects of products of vegetal origin have been performed, allowing the rational employment of these products (Queiroz et al., 2009).

Often, the antioxidant potential of either extracts with herbal actives or the pure isolated actives were evaluated, but very few reports are available on the antioxidant properties of final formulations. Gels are becoming more popular due to their easy application, better percutaneous absorption (when compared with other semisolid preparation) and resistance to the physiological stress caused by skin flexion, blinking and mucociliary movement, adopting the shape of applied area (Haneefa et al., 2010). Gels can be prepared with several polymers

like carbopol 940, hydroxyethyl cellulose and hydroxypropyl methylcellulose. Considering stability tests such as thermal stress, pH evaluation, viscosity and storage at different temperatures, carbopol 940 seems to be the most stable gelifying polymer (Queiroz et al., 2009).

In the present study, extracts from different parts of hawthorn (flower buds, flowers, unripe fruits, ripened fruits and over ripened fruits) were incorporated into hydrosoluble gels, which have been largely used in cosmetic products and as a dermatologic base, as they are easily dispersed, non-oily and can carry hydrosoluble active principles. The objective was the development of semi-solid formulas for topic use containing hawthorn extract and the study of their antioxidant activity.

2. Materials and methods

2.1. Standards and reagents

Gallic acid, catechin, imidazolidinyl urea, triethanolamine, 1,2-propanediol and poly(acrylic acid) (carbopol 940) were purchase from Sigma (St. Louis, MO, USA); 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.2. Samples

The material for analysis was collected, from small trees of the woodland, in sequence, during 2009 spring, summer and autumn, synchronized with the growth condition of buds, flowers and fruits, according to different gathering practices, the folk pharmacopoeia and local medicinal uses reported in the studied area (Bragança, Northeastern Portugal). Five different samples were considered: flower buds with top young leaves (corymbs); sprigs with both

leaves and flowers during anthesis (flower fully opened and functional); unripe fruits corresponding to flower senescence and stand out of the green pomaceous immature fruit; ripened fruits i.e. red pomes in late summer; over ripened fruits i.e. dark red, fleshy, sweet, chewy and coarse-textured pomes in late autumn.

Morphological key characters from the Flora Iberica (Castroviejo, 2001) were used for plant identification. Voucher specimens are deposited in the Escola Superior Agrária de Bragança herbarium (BRESA). Each sample was lyophilized (Ly-8-FM-ULE, Snijders, Holland) and kept at -20 ºC for subsequent use.

2.3. Samples preparation: extracts and hydrosoluble gels

For the extracts preparation, a fine dried powder (20 mesh; ~1 g) was stirred with 50 mL of ethanol (ethanolic extracts) or deionised water (aqueous extracts) at 25 °C and 150 rpm for 1 h, and filtered through Whatman No. 4 paper. The obtained residue was re-extracted under the same conditions. The combined ethanolic or aqueous extracts were evaporated at 35 °C under reduced pressure, re-dissolved in ethanol or water at 10 mg/mL, and stored at 4 °C for further evaluation of antioxidant activity.

For the gels preparation, a semi-solid base was prepared by adding 0.5 g Carbopol 940 to 20 mL of deionised water. The gel base was allowed to stand for 1 h after which 1 mL of triethanolamine and 5 mL of the hawthorn ethanolic or aqueous extract (1 mg/mL, corresponding to a final concentration of 100 μg/mL). Subsequently, citric acid (0.45 g), disodium EDTA (0.005 g), imidazolidinyl urea (0.1 g) and propylene glycol (7.5 g) was added. The final product was adjusted to 50 g by the addition of deionised water, and further submitted to the assays previously described for evaluation of antioxidant potential. A blank formulation (negative control) was also prepared in the same conditions but without incorporation of the extract. Three different commercial gels were also used as standards.

2.4. Antioxidant activity

2.4.1. DPPH radical-scavenging activity

This methodology was performed using an ELX800Microplate Reader (Bio-Tek Instruments, Inc). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts or gels (30 μL) and methanolic solution (270 μL) containing DPPH radicals (6×10^{-5} mol L^{-1}). The mixture was left to stand for 30 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against sample concentration.

2.4.2. Reducing power

This methodology was performed using the Microplate Reader described above. The different concentrations of the extracts or gels (0.5 mL) were mixed with sodium phosphate buffer (200 mmol L^{-1}, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm in the Microplate Reader described above. The concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 690 nm against sample concentration.

2.4.3. Inhibition of β-carotene bleaching

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 ml). Two millilitres of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40 ºC under vacuum and linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 ml) were added to the flask with vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred into test tubes containing solutions extract or gel with different concentrations (0.2 ml). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm (AnalytikJena 200-2004 spectrophotometer). β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100. The concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against sample concentration.

2.4.4. Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

Brains were obtained from porcine (*Sus scrofa*), dissected, and homogenized with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000*g* for 10 min. An aliquot (0.1 ml) of the supernatant was incubated with the solutions extract or gel with different concentrations (0.2 ml) in the presence of $FeSO_4$ (10 mM; 0.1 ml) and ascorbic acid (0.1 mM; 0.1 ml) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 ml), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 ml), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following

formula: Inhibition ratio (%) = [(A - B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively. The concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of TBARS formation inhibition percentage against sample concentration.

2.5. Determination of antioxidants content

For phenolics, an aliquot of the extract or gel solution (1 mL) was mixed with *Folin-Ciocalteu* reagent (5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g L^{-1}, 4 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 ^{o}C for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to calculate the standard curve (9.4×10^{-3}-1.5×10^{-1} mg mL^{-1}), and the results were expressed as mg of gallic acid equivalents (GAE) per g of sample.

For flavonoids, an aliquot (0.5 mL) of the extract solution was mixed with distilled water (2 mL) and subsequently with $NaNO_2$ solution (5%, 0.15 mL). After 6 min, $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min, thereafter, NaOH solution (4%, 2 mL) was added to the mixture. Immediately, deionised water was added to bring the final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510 nm. Catechin was used to calculate the standard curve (4.5×10^{-3}- 2.9×10^{-1} mg mL^{-1}) and the results were expressed as mg of catechin equivalents (CE) per g of sample.

2.6. Statistical analysis

For each extract three samples were analysed and also all the assays were carried out in triplicate. The statistical differences represented by letters were obtained through one-way analysis of variance (ANOVA) (SPSS software, v. 18.0). For each statistical significant

factor, means were compared using Tamhane's T2 test (all the distributions were heteroscedastic) with $\alpha = 0.05$, coupled with Welch's statistic The normality within groups and homogeneity of variances and of variance–covariance matrices, were checked using the Kolmogorov-Smirnov with Lilliefors correction, the Levene and M-Box tests, respectively.

Results and Discussion

As mentioned before, *Crataegus monogyna* Jacq., hawthorn, is well known for its important ethnopharmacological uses, commonly related with different disorders of the respiratory, circulatory and nervous systems and several skin conditions (Camejo-Rodrigues et al., 2003; Novais et al., 2004; Neves et al., 2009; Carvalho, 2010). Hence, *C. monogyna* extracts might have potential to be included in topical formulations with dermocosmetic applications. In order to compare the antioxidant potential of different botanical constituents extracted with low toxicity solvents, four *in vitro* chemical and biochemical assays were used: scavenging effects on DPPH radicals (measures the decrease in DPPH radical absorption after exposure to radical scavengers), reducing power (measures the conversion of a Fe^{3+}/ferricyanide complex to Fe^{2+}), inhibition of β-carotene bleaching (measures the capacity to neutralize the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models), and inhibition of lipid peroxidation in brain cells homogenates (measures the colour intensity of MDA-TBA complex). The results obtained for these assays, as well as those corresponding to bioactive compound contents, are presented in **Table 1**. The observed bioactivity of each botanical part seemed to be related with the used solvent and with the antioxidant assay (except for TBARS inhibition assay). Despite some composition-activity relationship effect, the found differences might be related with dissimilar yields: the most bioactive parts were the ones with minor yields, indicating that their extracts might contain less "impurities". Flower buds (FlB) and unripe fruits (UF) ethanolic extracts had the highest

DPPH scavenging activity; flower (Fl) ethanolic extracts had the maximum reducing power; UF and Fl aqueous extracts showed the highest β-carotene bleaching inhibition effect. Despite these good bioactivity indicators, the obtained values showed less antioxidant activity than those obtained with methanolic extracts (Barros et al., 2011), but the solvents tested in this work are more adequate to be incorporated in dermocosmetic products. The differences observed in the antioxidant activity were reflected in phenolics and flavonoids content, but a strong linear correlation was only found among DPPH scavenging activity and phenolics content (**Figure 1**). In general, ethanolic extracts proved to contain higher antioxidant compounds content than aqueous extracts (**Table 1**). Comparing plant parts, flower buds (for ethanolic extracts) and unripe fruits (for aqueous extracts) presented the highest amount of phenolics and flavonoids. Nevertheless, the values were lower than those obtained with methanolic extracts (Barros et al., 2011).

The next step was verifying if the antioxidant activity of each single extract was maintained after being included in the corresponding hydrosoluble gel. In order to optimize gel consistency, several carbopol 940 percentages were tested, being concluded that a 1% concentration allowed the best results. The colour of the prepared gels varied from light green (more evident in those prepared with ethanolic extracts) to light brownish. The prepared formulations presented an adequate non greasy texture and were promptly absorbed by the skin. For comparison purposes, the hydrosoluble gels were all prepared with 100 μg of extract/mL of gel formulation. Since the inclusion of parabens is nowadays poorly accepted by the consumers, imidazolidinyl urea was included as the antimicrobial component. Regarding pH evaluation (verified at room temperature, right after the preparation employing a digital pH meter Hanna Instruments) there were no significant alterations during the 90 days of observation in all analyzed gels, with pH values ranging between 5.5 and 6.5. The pH determination has high relevance to assess the stability of pharmaceutical formulations, since

alterations on pH values may occur due to impurities, hydrolysis, decomposition and error in the process (Queiroz et al., 2009). At this stage, it would not be advised to include different plant species or even different parts of the same plant in the same hydrosoluble gel formulation, since the safety and the efficiency of a phytotherapeutic drug must be defined for each product. These features depend on several factors, such as the methodology of collection of the extracts or the formulation and the pharmaceutical form of the final product (Queiroz et al., 2009).

After preparing the hydrosoluble gels, the same antioxidant assays applied to the extracts were performed in the hydrosoluble gel samples using also a reference control with all the components used in the hydrosoluble gel formulation, except the extract. The antioxidant activity value corresponding to the same concentration (100 μg of extract/mL) was also calculated for each isolated extract (**Table 2**). As it can be seen in **Figure 2**, the antioxidant activity measured in each hydrosoluble gel is very close to the value obtained for the isolated extract. In some cases (*e.g.*, gel with aqueous extract in DPPH), the values are overlapping, or even higher (gel with ethanolic extract in TBARS inhibition assay). Since it would be impractical to present graphs for all the assays, the complete data regarding the former comparison might be analysed in **Table 2**. It became evident that the inclusion of extracts in the prepared hydrosoluble gels caused very limited losses in their bioactivity. Thereby, the prepared formulation seemed to be suitable for the proposed rationale.

In addition to the antioxidant activity assays, the contents in phenolics and flavonoids were also compared (**Figure 3**). The prepared gels maintained a high percentage of the quantities of phenolics and flavonoids of the corresponding extracts included in their formulations. When compared to highly valued similar products available in the market (commercial samples forward denominated as A, B and C), the hydrosoluble gels obtained with different *C. monogyna* parts presented lower phenolics (A: 103±19; B: 53±2; C:26±2 mg GAE/g) and

flavonoids (A: 29±2; B: 29±3; C:23±2 mg CE/g) amounts. However, it should be reminded that the results obtained for the gels prepared in our laboratory were calculated subtracting the results of the "blank formulation" (negative control) that incorporates all the ingredients except the extract, while the commercial gels were measured without any subtraction. This observation is reinforced because the hydrosoluble gels prepared with FBE, FE and UFE showed higher DPPH scavenging activity and those prepared with FBE, FE, RFE, RFW, OFE and OFW had higher reducing power than the commercial formulations (DPPH (%): A- 26±2; B- 12±1; C- 2.1±0.4; reducing power ($Abs_{690\ nm}$): A- 0.22±0.01; B- 0.21±0.01; C- 0.20±0.01). In future works, the viscosity, extrudability and spreadability should be measured prior to the evaluation of the irritant reaction, which can be carried out with different instrumental techniques, such as the assessment of transepidermal water loss (TEWL) or erythema measurement.

Acknowledgements

The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT, Portugal) and COMPETE/QREN/EU for financial support to CIMO (strategic project PEst-OE/AGR/UI0690/2011). J.C.M. Barreira thanks to FCT, POPH-QREN and FSE for his grant (SFRH/BPD/72802/2010).

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Table 1. Antioxidant activity (EC_{50} values, µg/mL), phenolics (mg GAE/g) and flavonoids (mg CE/g) content of the extracts prepared from different parts of *Crataegus monogyna* (mean±SD, n=9).

		Yield (%)	DPPH scavenging activity[a]	Reducing power[a]	β-Carotene bleaching inhibition[a]	TBARS inhibition assay[a]	Phenolics[a]	Flavonoids[a]
FlB	Ethanolic	13±1 d	115±9 i	72±2 g	125±4 b	9.9±0.2 d	153±7 b	45±1 b
	Aqueous	32±2 b	415±4 f	453±21 c	74±1 c	61±1 c	98±1 d	23±1 cd
Fl	Ethanolic	13±1 d	167±6 h	110±4 f	153±7 c	9.0±0.3 d	170±3 a	31±2 c
	Aqueous	32±2 b	811±14 c	959±5 a	59±6 e	79±2 b	56±1 f	17±2 de
UF	Ethanolic	4.3±0.2 e	114±6 i	232±4 e	68±8 de	8±1 d	166±19 a	108±8 a
	Aqueous	12±1 d	323±13 g	466±16 c	57±11 e	72±6 b	118±1 c	54±3 b
RF	Ethanolic	25±2 c	629±28 d	276±5 d	74±21 d	9.1±0.3 d	83±2 e	51±14 b
	Aqueous	32±2 b	922±35 b	931±23 b	185±8 a	81±6 b	48±1 f	9±1 ef
ORF	Ethanolic	35±3 b	445±19 e	259±1 d	79±8 c	8.9±0.2 d	81±7 e	12±2 ef
	Aqueous	64±4 a	970±20 a	934±23 b	70±2 de	137±18 a	21±1 g	6±1 f
Homocedasticity[1]	*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Normal distribution[2]	*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.010	<0.001
One-way ANOVA[3]	*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

FlB- flower bud; Fl- flower; UF- unripe fruit; RF- ripened fruit; ORF- over ripened fruit

[1]Homoscedasticity among cultivars was tested through the Levene test

[2]Normal distribution of the residuals was evaluated using Kolmogorov-Smirnov with Lilliefors correction test (n>20).

[3]As $P<0.05$, the mean value of the evaluated parameter of at least one extract differs from the others and multiple comparison could be performed.

[a]Different letters in each column indicate mean values that differ significantly ($P < 0.001$). These differences were classified using Tamhane's T2 tests, since the homoscedasticity requirement was not fulfilled.

Table 2. Antioxidant activity of the hydrosoluble gels (prepared with incorporation of the extract at 100 μg/mL) and of the isolated extracts from different parts of *Crataegus monogyna* (at 100 μg/mL).

			DPPH inhibition (%)	Reducing Power ($Abs_{690\ nm}$)	β-Carotene bleaching inhibition (%)	TBARS inhibition (%)
FlB	Ethanolic	Hydrosoluble gel	33±4	0.7±0.2	24±3	87±3
		Extract	43±3	0.66±0.01	27±1	90±1
	Aqueous	Hydrosoluble gel	11±1	0.051±0.0001	39±1	62±1
		Extract	14±1	0.056±0.005	46±2	69±1
Fl	Ethanolic	Hydrosoluble gel	28±3	0.50±0.01	30±1	81±2
		Extract	31±1	0.50±0.03	35±1	90±1
	Aqueous	Hydrosoluble gel	6±1	0.13±0.01	39±1	47±4
		Extract	14±1	0.146±0.004	46±1	57±2
UF	Ethanolic	Hydrosoluble gel	28±4	0.14±0.04	60±1	69±1
		Extract	45±2	0.15±0.01	87±1	77±3
	Aqueous	Hydrosoluble gel	20±3	0.04±0.01	48±2	26±1
		Extract	20±1	0.051±0.002	65±6	39±2
RF	Ethanolic	Hydrosoluble gel	15±1	0.33±0.01	37±4	74±1
		Extract	14±1	0.22±0.03	45±1	84±2
	Aqueous	Hydrosoluble gel	9±1	0.45±0.04	40±1	50±2
		Extract	10±1	0.07±0.01	40±1	56±2
ORF	Ethanolic	Hydrosoluble gel	15±1	0.44±0.04	35±1	67±1
		Extract	15±2	0.23±0.01	41±1	86±4
	Aqueous	Hydrosoluble gel	5±1	0.37±0.04	38±2	24±2
		Extract	16±3	0.064±0.004	41±1	28±1

The antioxidant activity of the isolated extracts was obtained by interpolation in the gaphs of antioxidant activity *versus* extract concentration. FlB- flower bud; Fl- flower; UF- unripe fruit; RF- ripened fruit; ORF- over ripened fruit.

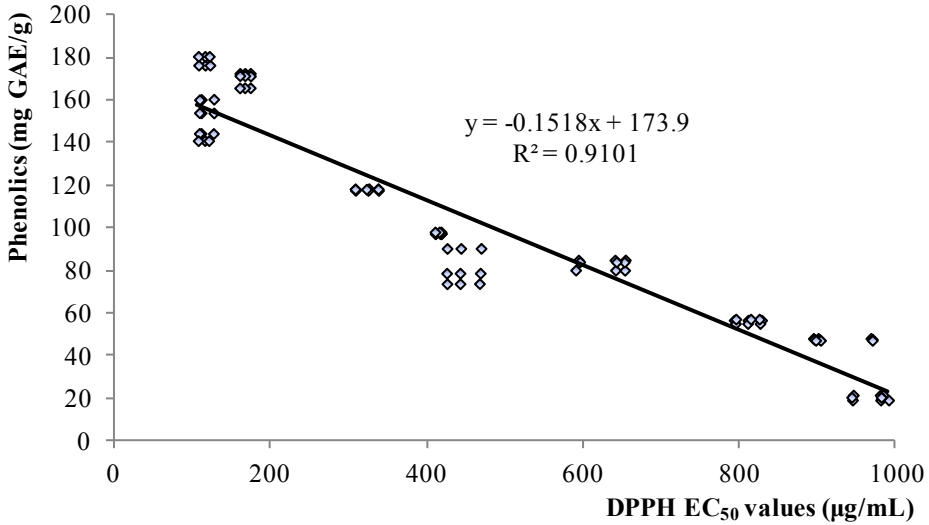

Figure 1. Correlation between DPPH radical scavenging activity and phenolics content (all the results were used).

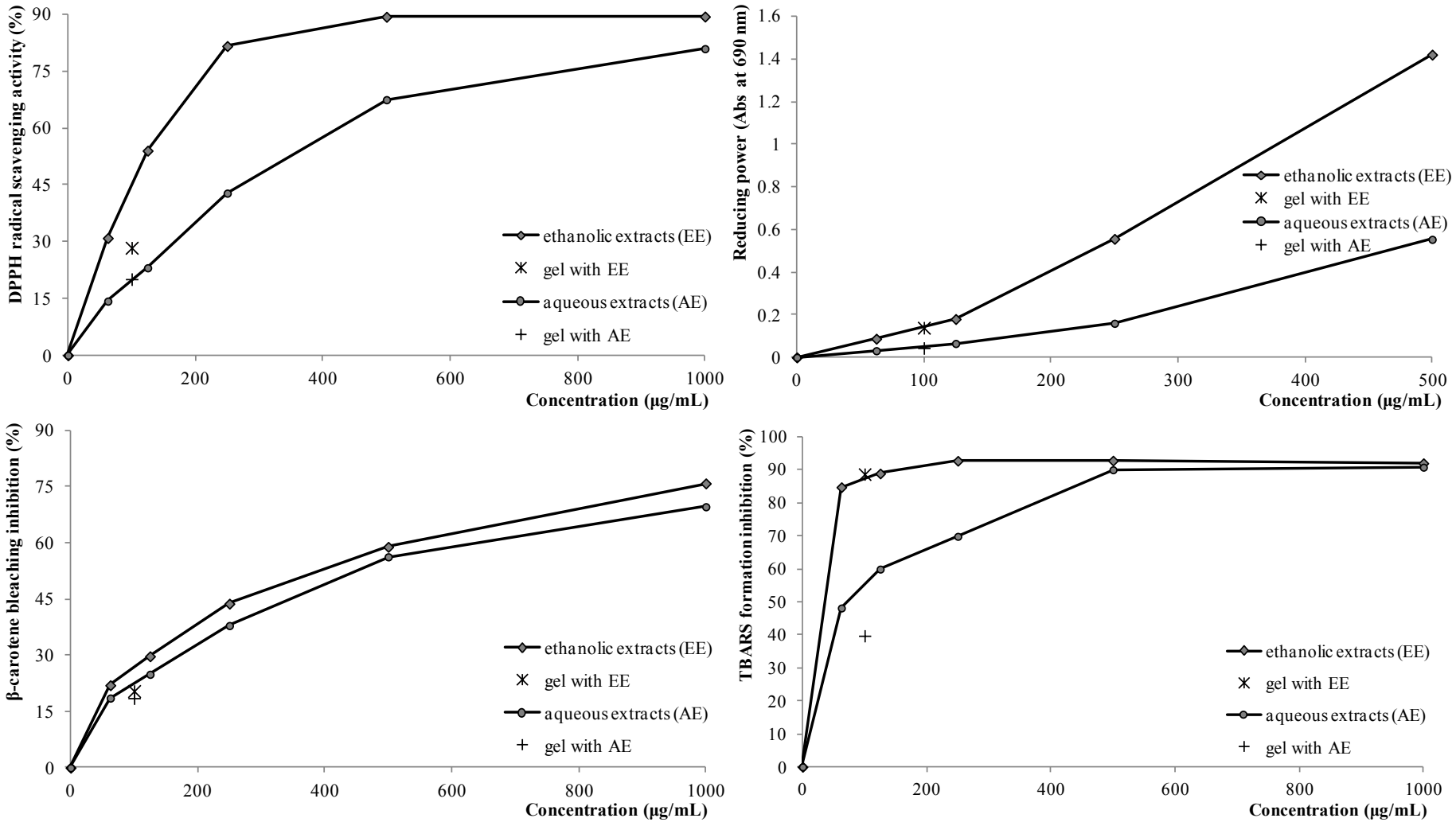

Figure 2. Antioxidant activity of *Crataegus monogyna* unripe fruits ethanolic and aqueous extracts. Comparison with the value obtained for each prepared hydrosoluble gel (with incorporation of the extract at 100 µg/mL).

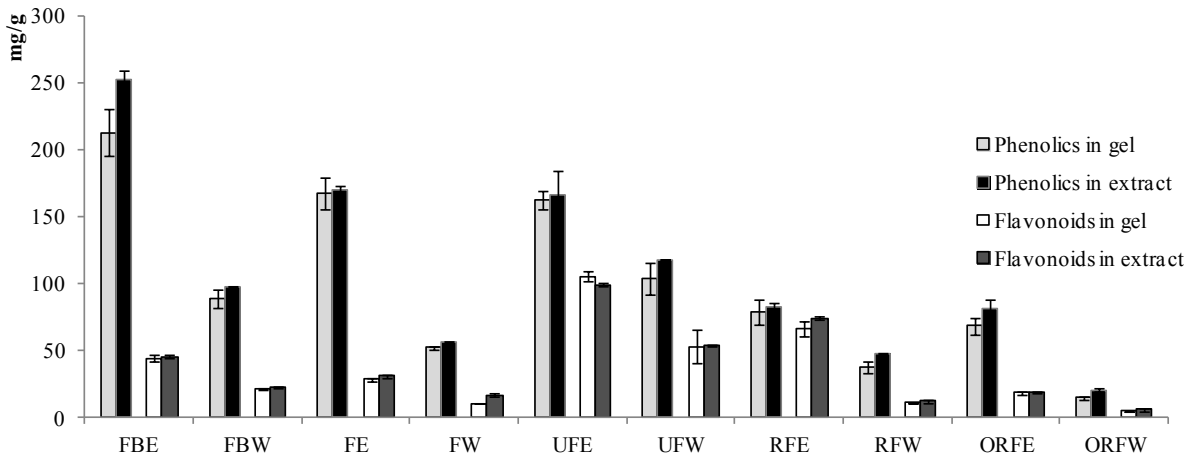

Figure 3. Phenolics and flavonoids content in the prepared hydrosoluble gels (with incorporation of extract at 100 µg/mL) and in isolated extracts at 100 µg/mL. FBE- flower buds ethanolic extract; FBW- flower buds aqueous extract; FE- flowers ethanolic extract; FW- flowers aqueous extract; UFE- unripe fruits ethanolic extract; UFW- unripe fruits aqueous extract; RFE- ripened ethanolic extract; RFW- ripened fruits aqueous extract; ORFE- over ripened ethanolic extract; ORFW- over ripened aqueous extract.