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Comparison of the antioxidant effect and phenolic profile of two *Crataegus* extracts

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Abstract

This study was designed to evaluate the phenolic profiles and antioxidant potentials of two commercial *Crataegus monogyna* Jacq samples, which are commonly used for various medicinal purposes. One of the samples supplied from the pharmacy and the other sample supplied from the herbalist shop. To analyze phenolic contents of samples Total Phenolic Content, Total Flavonoid Content and Total Phenolic Acid Content Assays were carried out. Further hyperoside contents, which is one of the major component of the genus, were determined by HPTLC method. Antioxidant bioactivities were established by using CUPRAC (Cupric Reducing Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power), DPPH radical scavenging activity and TOAC (Total Antioxidant Capacity) methods. The comparison between two samples showed that the pharmacy product has higher phenolic ingredients than herbalist shop, consequently, has higher antioxidant bioactivity.

Keywords: *Crataegus monogyna*, pharmacy, hyperoside, antioxidant

Introduction

Crataegus (Hawthorn) species have traditional and widespread use as an alternative treatment or prevention in the many cardiovascular diseases such as hypertension, angina, arrhythmia, and early stages of heart failure due to their high phenolic content [1]. Besides, anti-inflammatory, antiallergic, antiarthritic, hypolipidemic and antidiabetic activities have been reported and those biological activities are thought to arise from the plant's phenolic composition [2].

Leaves, flowers, berries of the genus *Crataegus* contain high amounts of flavonoids, procyanidins, phenolic acids, and few other secondary metabolites [3]. The most prevalent ones among these compounds are, procyanidins (procyanidin B_2, procyanidin B_5, and procyanidin C_1), flavonoids (epicatechin, hyperoside, quercetin, rutin, and isoquercitrin), and triterpenoids (ursolic acid, corosolic acid, oleanolic acid, and maslinic acid) [4].

The objective of the present study was to investigate the difference in antioxidant potential and phenolic content between two hawthorn (*Crataegus monogyna*) samples, which were obtained from different stores, pharmacy and herbalist. Methanolic extract of leaves evaluated for their antioxidant potential by using CUPRAC (Cupric Reducing Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power), DPPH radical, scavenging activity, TOAC (Total Antioxidant Capacity) methods. Total Phenolic Content, Total Flavonoid Content and Total Phenolic Acid Content Assays carried out to detect and quantify phenolic profile. Hyperoside content was determined by HPTLC method as well.

Materials and Methods

Plant Materials

C. monogyna samples were obtained from pharmacy and herbalist shops. The plant materials were identified by one of us (HB).

Extraction and preparation of plant samples

Grinded *C. monogyna* samples were macerated with 400 mL of methanol (MeOH) for three days at room temperature. MeOH was evaporated until dryness by using rotavapory and the remaining extract was lyophilized. The extract yields of Hawthorn MeOH extracts from pharmacy and herbalists were calculated as 15.80 and 11.75 %, respectively. For the quantitative assessment of phenolic profile and estimation of antioxidant capacity 1 mg/mL of *C. monogyna* MeOH extracts were prepared in MeOH. The sample solutions were filtered using 45 µm filters before tests.

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Quantitative assessment of the phenolic profile

Total phenolic content

The assessments of the total phenolic content of the samples were performed according to the method Singleton and Rossi (1965) [5]. Diluted samples were inserted into the mixture of Na_2CO_3 (20%) and Folin Ciocalteu reagent (diluted with H_2O). At the end of the incubation period at 45 °C, the absorbance of the mixtures was measured at 765 nm. The results were expressed as gallic acid equivalents (GAE) per g dried extract (DE).

Total flavonoid content

The total flavonoid content of the samples was calculated according to the method described by Celep et al. (2012) [6]. Sample dilutions were combined with a mixture of A_1C_{l3} and sodium acetate and left for incubation at room temperature for 30 min. The absorbance was read at 415 nm. Total flavonoid content was expressed as mg quercetin equivalents (QE) per g DE.

The total phenolic acid content

The total phenolic acid content of the samples was measured spectrophotometrically at 490 nm (Mihailovic et al., 2016) [7]. This method relies on the formation of a complex due to the interaction of sodium nitrite-sodium molybdate with phenolic acids. The results were estimated as caffeic acid equivalents (CAE) in per g DE.

HPTLC quantification of hyperoside

Hyperoside contents were measured by using the previously published method Cretu et al. (2013) [8]. The standard solution of hyperoside (250 µg/mL) was prepared in MeOH, and 50 mg of total *Crataegus* extracts were dissolved in 10 mL MeOH. Each extract was filtered through a 0.45 µm syringe filter. 8 µL of *C. monogyna* extracts and 3 µL to 13 µL of standard hyperoside solution were applied in triplicate. The sample and standard solutions were spotted in the form of bands with an 8 mm length on silica gel glass HPTLC plates 60 F254 with Camag Automatic TLC Sampler IV. A constant application rate was applied and the spaces between the tracks were 10 mm. The mobile phase was ethyl acetate:acetic acid:formic acid:water 10:1.1:1.1:2.3 (v/v/v/v%). Developments were carried out in Camag Automatic Developing Chamber (ADC-2). Chamber was saturated for 20 min and the plate is preconditioned for 5 min before the development. The humidity is controlled by ADC-2 using MgC_{l2} (33% RH) for 10 minutes. The plate was sprayed with NP reagent using Camag Derivatizer and then heated for 5 min by using Camag plate heater at 100 ℃ after the development of 70 mm. The densitometric screening was performed by using Camag TLC Scanner IV and VisionCATS software in fluorescence mode after derivatization with 2-aminoethyl-diphenyl borinate reagent (NP) by Camag Derivatizer at 330 nm. The slit dimension was kept at 5×0.2mm, micro and the scanning speed was set at 20 mm/s. Standard contents were afforded by comparing AUCs with the calibration curve of standards. The coefficient of variation (CV %) is under 1.00 and the correlation coefficient (R) of the calibration curve was above 0.998. The presence of standards in extracts was assured by comparison of both retention factors (Rf) and overlaying UV spectra of each extract and standards.

Estimation of antioxidant activity based on metal-related activity

Cupric reducing antioxidant capacity (CUPRAC)

The total determination of the CUPRAC activity of the samples was performed according to the modified method of Apak et al. (2004) [9]. The same volumes of neocuproine, ammonium acetate buffer and $CuSO_4$ were separately mixed. After the addition of samples, the mixture was incubated for 1h, and the absorbance was measured at 450 nm. The results were expressed as mg ascorbic acid equivalent (AAE) per g DE.

Ferric reducing antioxidant power (FRAP)

The spectrophotometric method applied was described previously by Benzie and Strain (1996) [10]. Properly diluted samples were mixed with FRAP reagent, and the volume was adjusted to 0.3 mL. After incubating 30 min, the absorbance was read at 593 nm. BHT was used as a reference compound. The results were expressed as mM $FeSO_4$ per g DE.

Determination of total antioxidant capacity by phosphomolybdenum method

The method described by Prieto, Pineda, and Aguilar (1999) was employed for the determination of total antioxidant capacity [11]. Sample solutions were added to the reaction mixture composed of ammonium molybdate, sulfuric acid, and sodium phosphate monobasic. Following the incubation at 95 °C for 1.5 h, the absorbance was read 695 nm. Total antioxidant capacity was expressed as mg AAE per g DE.

Estimation of antioxidant activity based on free radical-scavenging activity

DPPH radical-scavenging activity

DPPH radical-scavenging activity test was conducted according to the method defined earlier by Celep et al. (2013) [12]. Freshly diluted samples were separately mixed with a 100 µM methanolic DPPH solution. The mixture was kept at room temperature in the dark, and the absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as a reference compound.

Results

Quantification of the phenolic profile of the samples

Total phenolic, total phenolic acid, and flavonoid contents of two *Crataegus* samples were shown in Table 1.

Table 1. Spectrophotometric determination of phenolic profile of *Crataegus monogyna*

Analysis	CMP	CMH
Total phenolic content^{A}	199.51±0.21	**113.82±0.07**
Total flavonoid content^{B}	86.69±0.02	29.516±0.00
Total phenolic acid content^{C}	57.00±0.00	24.46±0.01

^{A} Results were expressed as the mean of triplicates ± standart deviation (S.D.) and as mg gallic acid equivalents (GAE) in 1 g sample.
it should be added in beginning and A should be in superscript.
^{B} Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg quercetin equivalents (QE) in 1 g sample.
^{C} Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg caffeic acid equivalents (CAE) in 1 g sample.

HPTLC analysis of hyperoside

Hyperoside contents of each extract were quantified by using HPTLC. The Rf value was found to be 0.24 for hyperoside (Figure 1). Quantification of standards was afforded by comparing AUCs with the calibration curve of standards (Figure 2). The authentication of standard compounds in plant samples was verified by comparison of Rf values and their UV spectra (Figure 3).

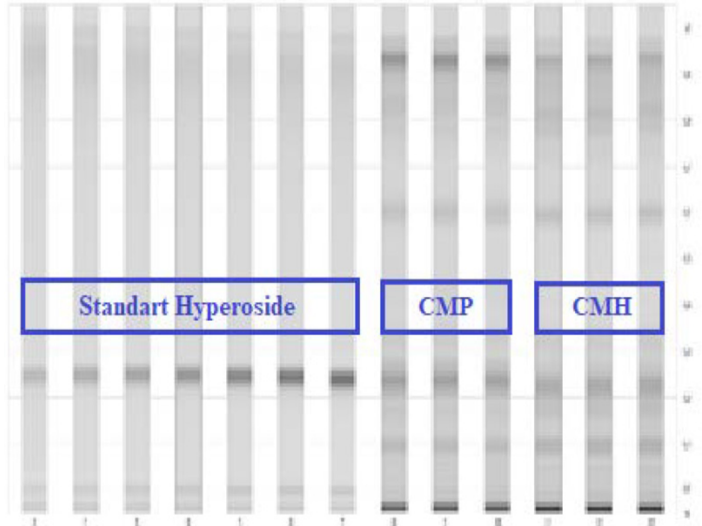

Figure 1. Separation and Rf value of standard hyperoside in *Crataegus* extracts

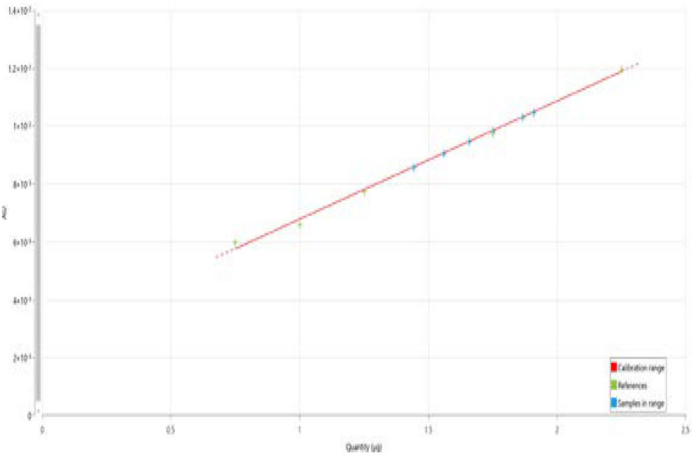

Figure 2. Calibration curve for hyperoside and contents of *Crataegus* extracts on the calibration curve

The results of the HPTLC study were given in Table 2.

Table 2. Quantification data for hyperoside from *Crataegus* extracts

Extract	Hyperoside (w/w %)	CV%
CMP*	4.602	**0.03**
CMH**	3.882	1.46

*: Crataegus monogyna from the pharmacy. ** Crataegus monogyna from the herbalist. CV: coefficient of variation.

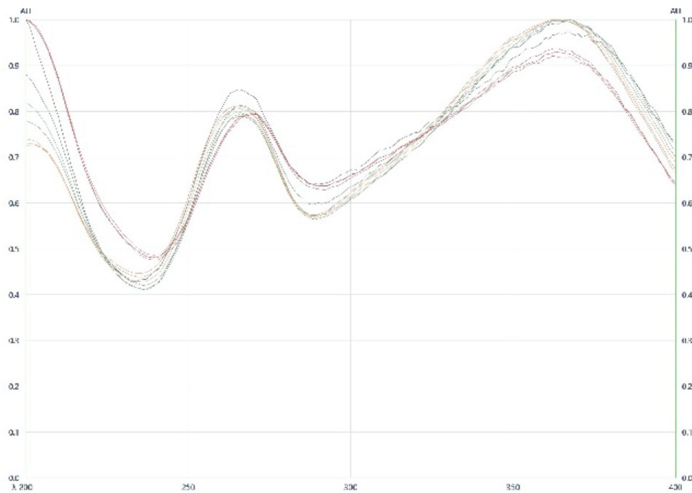

Figure 3. Overlapped UV spectra of hyperoside standard and *Crataegus* extracts

Estimation of antioxidant activity

To evaluate the antioxidant spectrum of *Crataegus* species, their effect on DPPH radical and metals were investigated. The results are summarized in Table 3.

Table 3. Quantification data for hyperoside from *Crataegus* extracts

Samples/Tests	DPPH[A]	FRAP[B]	CUPRAC[C]	TOAC[c]
CMP	554.92	4.31±0.02	520.26±0.00	5217.24±0.022
CMH	547.1	1.56±0.09	389.14±0.03	4491.15±0.08

1) The IC_{50} value of the reference compound "BHT" in DPPH scavenging activity is found to be 350±10 μg/mL. 2) FRAP activity of the reference compound "BHT" is found to be 4.24 ± 0.48 mM $FeSO_4$ eq. in 1 g sample.
[A] Results were expressed as the mean of triplicates ± standard deviation (S.D.), and DPPH activity was expressed as IC_{50} in μg/mL equivalents.
[B] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as Mm $FeSO_4$ equivalents in 1 g sample.
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.

Discussion

Herbal medicine products have become progressively popular, but herbal products may be inefficient even may be hazardous if they are used unconsciously. To prevent such problems, medicinal plants should be standardized and controlled in terms of their chemical ingredients and other qualities [13].

Crataegus species are one of the most commonly used herbal medicines owing to their rich phenolic ingredients. Plenty of research has been carried out about the antioxidant potential of phenolic molecules [14]. Through the agency of high phenolic contents, the genus is predominantly used as an antioxidant source and a treatment agent for the other oxidative diseases[1-2]. Therefore it is important to know the phenolic profile and antioxidant activity of each *Crataegus* product to be used.

In the present study, two extracts of *Crataegus monogyna* compared in point of their phenolic contents and antioxidant activities. The results showed that the drug which bought from the pharmacy (CMP) has more phenolic, flavonoid, phenolic acid content than

herbalist product (CMH).

HPTLC analysis revealed that CMP contained by far the highest hyperoside among the tested extracts.

Somewhat difference established in antioxidant potential between two samples as well.

CMP showed the highest FRAP and CUPRAC activity and, likewise, the highest antioxidant capacity according to the results.

IC_{50} values were calculated for the comparison of DPPH radical, sweeping capacities, and calculations showed that there is no significant difference between CMP and CMH.

In the previous, there are many investigations about phytochemical and antioxidant analysis of *Crataegus* species [15-18]. In the current study, two commercial *Crataegus monogyna* extracts compared at this point, and results showed that pharmacy product displays better properties than herbalist shop product. It could be concluded that individual features, agricultural properties, storage conditions and many other factors may influence the quality of the herb.

Conclusion

Two extracts of *Crataegus monogyna* compared in point of their phenolic contents and antioxidant activities. The results showed that the drug which bought from the pharmacy (CMP) has more phenolic, flavonoid, phenolic acid and hyperoside content than herbalist product (CMH).

Somewhat difference established in antioxidant potential between two samples as well. Antioxidant activity determined by CUPRAC, FRAP, TOAC, DPPH methods, and CMP has higher antioxidant activity than CMH according to the results.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
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Ethical approval
The author confirms that this article content is not required to have consent of ethics.

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