Journal of Agriculture and Environmental Sciences
December 2018, Vol. 7, No. 2, pp. 131-142
ISSN 2334-2404 (Print) 2334-2412 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jns.v7n2a14
URL: https://doi.org/10.15640/jns.v7n2a14

# Bioactive compounds in aqueous infusions of dietary supplements and herbal blends containing dried hawthorn fruits or hawthorn inflorescences (*Crataegus* spp.)

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**Abstract**

The aim of this study was the preliminary analysis of micronutrient and phytochemical composition as well as antioxidant activity of aqueous extracts obtained from dietary supplements and herbal blends containing dry hawthorn fruits (HF) and hawthorn inflorescences (HI). The study included 15 samples. Total phenols (TPC) and flavonoids content (TFC) of aqueous extracts were determined using colorimetric assay. The antioxidant activity was analyzed by using DPPH and ABTS methods. To analyze the concentration of citric and L-malic acid were used enzymatic analysis, while to analyze of tartaric acid was used colorimetric method. Ascorbic acid and oxalic acid were determined using titration method. Mean TPC and TFC for aqueous extracts with dry HI is nearly 2.5 and 4.0 times higher than in extracts with dry HF, respectively. Results shows that TPC, TFC is positively and highly correlated with fragmentation degree of dry HF. High correlation was observed between TFC and DPPH and ABTS values, while no correlation observed between TPC and antioxidative activity with DPPH and ABTS assays of HF. The analysis of principal component analysis demonstrates different clusters based on the chemical composition separating aqueous extracts with HF from the HI.

**Keywords:** Crataegus monogyna, hawthorn fruits, hawthorn inflorescences, leaves, PCA

## Introduction

Hawthorn (*Crataegus* spp.) is comonly used as medicinal and food materials in China and European countries in the prevention of cardiovascular and hepatic diseases. It was proved that administration of hawthorn extracts to patients contributes to the reduction of total cholesterol, LDL and has an effect on increasing HDL cholesterol (Przybylska et al. 2018). The results of Liu et al. (2018 a) suggests that hawthorn polyphenol extract can be prevent UVB radiation-induced skin photoaging. According to data from the GUS-State Statistics Office of Poland, 157 tons of fresh hawthorn fruit are collected by local people and sold for herbal raw materials in Poland. The largest amount of fresh hawthorn fruit is gathered in the Podlaskie (86 tons), Lubelskie (25 tons) and Podkarpackie (24 tons) voivodeship (GUS-State Statistics Office). In Latin America (Chile), in 2005 hawthorn (*Crataegus monogyna*) was collected in a quantity of 93 tons from 1800 ha area (Censkowsky et al. 2007). Referring to recommendation of European Pharmacopaeia dietary supplements may contain dried hawthorn fruits (HF) of *Crataegus monogyna* Jacq. (Lindm.) or *Crataegus laevigata* (Poir.) DC. (syn. *C. oxyacantha* L.) or their hybrids or a mixture of these all fruit (Przybylska & Bazylak 2017). In Poland, the most numerous group of *Crataegus* spp. is *Crataegus monogyna* and *Crataegus laevigata*. In China the major species are *C. pinnatifida*, *C. brettschneideri* or *C. scabrifolia* while in Finland is *C. grayana* (Yang & Liu 2012). A subject of many studies are antioxidant properties and the content of bioactive compounds in fresh hawthorn fruits broken from wild trees (Pliszka et al. 2016, Liu et al. 2011). Plant material used for the production of dietary supplements and herbal blends is characterized by high variability due to climatic conditions, harvest time and storage conditions. Technological processes (drying, packaging, transport, crushing) used for the production of dietary supplements and herbal blends can change the content of the active compounds in hawthorn fruits (HF) and hawthorn inflorescences (HI) (Przybylska et al, 2018).

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From the point of view of the consumer, information on the content and chemical composition of bioactive compounds in aqueous extracts of dietary supplements containing hawthorn fruits and hawthorn inflorescences are still very limited.

The aim of this study was to analysis the total phenols (TPC) and total flavonoids content (TFC), antioxidant activity and ascorbic, citric, malic, tartaric and oxalic acid content in aqueous extracts of commercial dietary supplements and herbal blends containing dry HF and HI purchased in Poland. The purpose of this study was to assess the impact of the degree of fragmentation of dry hawthorn fruits (HF) and hawthorn inflorescence (HI) on the content of analyzed chemical compounds in water infusions.

**Materials and methods**

Chemicals

Sodium carbonate, sodium nitrate, aluminium chloride hexahydrate, sodium hydroxide, methanol, potassium persulphate, oxalic acid, potassium permanganate, calcium chloride dihydrat, acetone, sulfuric acid were purchased from POCH (Gliwice, Poland). DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ABTS (2,2'azinobis-(3-ethylbenzthiazoline-6-sulphonic acid), phosphate-buffered saline (PBS), 2,6-dichloroindophenol sodium salt hydrate, (±)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), (+)-catechin hydrate, gallic acid, Folin-Ciocalteu's phenol reagent came from SigmaAldrich (Steinheim, Germany). Purified water used to prepare the solution was conductivity of 0.10 μS $cm^{-1}$ (HLP Smart 2000, Hydrolab, Poland).

Samples

Commercial dietary supplements and herbal blends containing dry HF and HI were purchased from supermarkets, pharmacy stores in Bydgoszcz (Poland) and purchased online. Products (n = 15) came from organic farming (n = 1) and conventional farming (n = 14) located in various regions of Poland. The analyzed products contained dry HF (100%), products containing HF and different part of medicinal plants i.e. hawthorn inflorescence, hibiscus flower, apple fruit, chokeberry fruit, rosehip and products containing only dry HI. The material differed in the degree of fragmentation. In the study was used a representative sample taken from each of the analyzed products (Table 1).

**Table 1. Characteristics of the analyzed samples.**

| Sample | BD*** | Manufacturer | Voivodeship **** | Form |
|---|---|---|---|---|
| Hawthorn fruits (HF) | | | | |
| FG1 | 0.41 | Kawon | Wielkopolskie | single package (50.0 g) |
| FG2 | 0.56 | Kawon | Wielkopolskie | sachets (3.0 g) |
| FG3 | 0.48 | Kawon | Wielkopolskie | sachets (3.0 g) |
| FG4 | 0.38 | Flos | Łódzkie | single package (50.0 g) |
| FG5 | 0.38 | Flos | Łódzkie | single package (50.0 g) |
| FG6 * | 0.37 | Dary Natury | Podlaskie | single package (100.0 g) |
| FG14 | 0.22 | Skarby Natury | Lubuskie | single package (50.0 g) |
| FG15 | 0.29 | Internet supplier | No data | single package (150.0 g) |
| FG7 ** | 0.51 | Bifix | Łódzkie | sachets (2.0 g) |
| FG13 ** | 0.46 | Malwa | Lubuskie | sachets (2.0 g) |
| Hawthorn inflorescence (HI) | | | | |
| IG8 | 0.19 | Flos | Łódzkie | single package (50.0 g) |
| IG9 | 0.22 | Kawon | Wielkopolskie | single package (50.0 g) |
| IG10 | 0.23 | Herbapol | Małopolskie | sachets (2.0 g) |
| IG11 | 0.28 | Herbapol | Małopolskie | sachets (2.0 g) |
| IG12 | 0.16 | Kawon | Wielkopolskie | single package (50.0 g) |

* - products with an ecological certificate, ** - FG7 – hawthorn fruit (40 %), apple fruit, chokeberry fruit, hibiscus flower, citric acid and FG13 – hawthorn fruit (51 %), hibiscus flower, hawthorn inflorescence (10 %), rosehip), *** - bulk density [g/mL], **** - grouping by manufacturer's headquarters.

**Analytical procedure**

The conditions of extraction were similar to the conditions used by the consumer at home. For water extraction, one gram of the sample was infused with 50.0 mL freshly boiled deionized water for 5 min (under cover). Next, the extract was filtered with use of a filter paper and a Chromafil PES-45/25 syringe filter (Macherey-Nagel, Germany). The bulk density (BD) of the tested dietary supplements and herbal blends containing dry HF and HI was determined according to the method of Ogrodowska et al. (2011). The results were expressed in g $mL^{-1}$.

The total phenols content (TPC) in the aqueous extract of dietary supplements and herbal blends containing HF and HI was determined with Folin-Ciocalteu reagent according to the method of Turkmen et al. (2007). One milliliter of the aqueous extraction was mixed with 1.0 mL of 3-fold-diluted Folin-Ciocalteu reagent and 2.0 mL 35 % $Na_2CO_3$. The mixture was shaken thoroughly and 2.0 mL of deionized water was added. The mixture was allowed to stand for 30 min. The absorbance was measured at $\lambda$ = 700 on UV-VIS spectrophotometer type 1300 (Zeiss, Jena, Germany). The determination of TPC was carried out in a triplicates and calculated from the calibration curve obtained with gallic acid GAE. The results were expressed as mg GAE eq. 100 g dry weight (DW). The total flavonoid content (TFC) in the aqueous extracts prepared from dry HF and HI was measured with aluminum chloride colorimetric assay according to the method of Atanassova et al. (2011). One milliliter of aqueous extraction was mixed with 4.0 mL deionized water, 0.3 mL 5% $NaNO_2$ and 0.3 mL 10% $AlCl_3$ after five min. At the sixth minute, 2.0 mL 1 M NaOH was added and the total volume was made up to 10.0 mL with deionized water. The absorbance was measured at $\lambda$ = 510 nm on UV-VIS spectrophotometer. The determination of TFC was carried out in a triplicates and calculated from the calibration curve obtained with (+)-catechin (CE). The results were expressed as mg of (+)-catechin (CE) eq. 100 g DW. The antiradical activity (DPPH) was determined by the Atanassova et al. (2011) method. One mililiter of aqueous extracts and Trolox (TE) standard solution were mixed with 4.0 mL 0.004% methanol stock solution of DPPH in. After vortex and 60 min. incubation in a dark place in room temperature the absorbance was read against a blank at $\lambda$ = 517 nm on UV-VIS spectrometer. The DPPH assay was done as carried out in a triplicates and calculated from the calibration curve obtained with Trolox (TE). The results were expressed as mg of TE eq. per 100 g (DW) and mM of TE eq. per 100 g DW. The percent inhibition of the $DPPH^{\cdot}$ or $ABTS^{\cdot+}$ radical was calculated using the following formula:

$$I_{DPPH\cdot}\ \% \text{ or } I_{ABTS\cdot+}\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where: $A_{sample}$ - the mean absorbance values in the presence in the extract, $A_{blank}$ - absorbance of the control solution (DPPH or ABTS stock solution respectively). The extract concentration inducing 50% of DPPH radicals inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against extract concentration. For the ABTS assay, the procedure followed the method of Re et al. (1999) with some modification. ABTS stock solution (7.0 mM) was prepared by adding 3.3 mg potassium persulfate ($K_2S_2O_8$), 19.5 mg of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 7.0 mL phosphate-buffered saline (PBS, pH 7.4). The mixture was kept in the dark at room temperature for 12 - 16 h. The ABTS radical cation ($ABTS^{\cdot+}$) was prepared by diluting the stock solution using deionized water so that the absorbance at $\lambda$ = 734 nm was measured A = 1.0. Next, 0.03 mL of aqueous extracts and TE standard solution were added to 0.97 mL stock solution and absorbance was measured on UV-VIS spectrophotometer. The ABTS assay was done as carried out in a triplicates and calculated from the calibration curve obtained with TE. The results were expressed as mg of TE eq. per 100 g DW and mM of TE eq. per 100 g. To analyze the concentration of citric acid (citrate, CIAC) and L-malic acid (L-malate, MAAC) in aqueous extracts prepared from dry supplements and herbal blends containing HF and HI, an enzymatic analysis was performed using the K-CITRIC and K-MALQR diagnostic kits were used respectively obtained from Megazyme (Brey, Ireland). To analyze of tartaric acid content (tartrate, TAAC) in aqueous extracts colorimetric method using the K-TART kit (Megazyme, Ireland) was used. All steps of the analysis were carried out in accordance with the manufacturer's assay procedure. The absorbance of analyzed solution was measured at $\lambda$ = 340 nm for citric acid and L-malic acid and $\lambda$ = 505 nm for tartaric acid on UV-VIS spectrophotometer type 1300 (Zeiss, Jena, Germany). The results were expressed as mg per 100 g DW. Ascorbic acid (ASAC) content in aqueous extracts was determined using the 2,6-dichlorophenol-indophenol titration method (Tillmans method) followed the method Kabaskalis et al. (2000). The concentration of the titrant (Tillmans reagent) was 0.005 mg $mL^{-1}$. Five milliliters of the aqueous extract of dry dietary supplements and herbal blends was dissolved in 45.0 mL of the 3.0% oxalic acid ($C_2H_2O_4$) in 50.0 mL volumetric flask.

After that, the solution was vortexed and 10.0 mL of solution was collected three times for analysis. Oxalic acid (OXAC) content in aqueous extracts was determined by manganometric titration using 0.002 M $KMnO_4$ standard solution (titrant) followed to the method Sperkowska & Bazylak (2010). After filtration, 2.5 mL of aqueous extract was poured into a centrifuge tube with 1.0 mL 25% $CaCl_2$ and 2.0 mL acetone in centrifuge tube. The mixture was vortexed and frozen for 30 min. at -20 °C. After this time, the solution was centrifuged at 3500 × g for 5 min. The washed precipitate was dissolved in 5.0 mL of 10% $H_2SO_4$. The results were expressed as mg per 100 g dry DW. Total acidity (ACID) in aqueous extracts was measured by potentiometric titration with use of a semi-automatic analyzer DL-22 (Mettler Toledo, Switzerland). The analyze solution was prepared by dissolving 5.0 mL of aqueous extract of dietary supplements and herbal blends containing HF and HI in 50.0 mL of deionized water (HLP smart 2000, Hydrolab, Poland). The results were expressed as citric acid eq. per 100 mL.

**Statistical analysis**

The result was presented as mean ± standard deviation and three replicate of each sample. Parametric t-Student test was used to compare differences of means ($\alpha = 0.05$). Significance was assumed for $p < 0.05$. In order to describe the relationships of multidimensional data arrays between values of variables the correlation matrix and principal component analysis (PCA) were calculated. All statistical analyses were performed using STATISTICA v.13 (StatSoft, USA).

**Results and discussion**

Total phenols content (TPC) and total flavonoids content (TFC). According to the results, the range of TPC in aqueous extracts prepared from HF and HI was 15.91 ÷ 1206.79 mg GAE $100g^{-1}$ DW and 937.13 ÷ 1444.12 mg GAE $100g^{-1}$ DW, respectively (Figure 1). Mean TPC for aqueous extracts with HI is nearly 2.5 times higher compared with an extracts from HF.

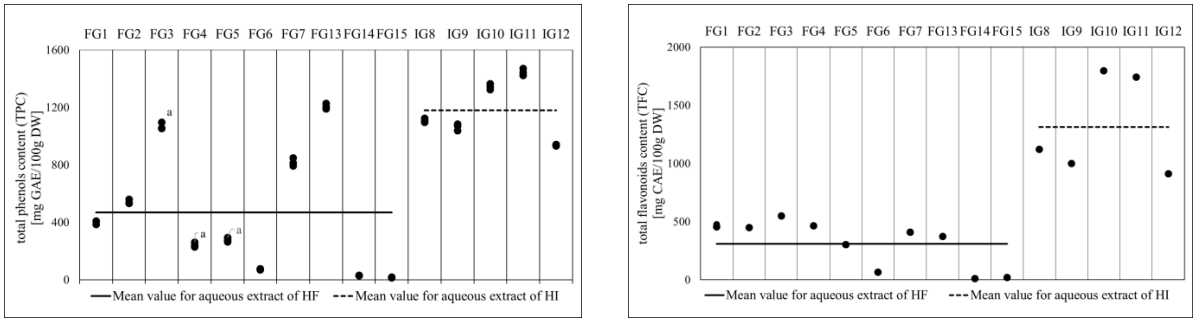


**Figure 1. Total phenols content (TPC) and total flavonoids content (TFC) of aqueous extracts obtained from dry HF (FG1 – FG15) and HI (IG8 – IG12). The small letters indicate the no statistically significant differences ($p > 0.05$) between samples tested**.

In the group of products containing hawberries, the highest TPC were determined in multi-component sample FG7 and FG13 (817.16 and 1206.79 mg GAE 100g $DW^{-1}$). This is probably related to the addition parts of different plants to herbal blends (apple fruit, chokeberry fruit, hibiscus flower) by the manufacturer. According to Mak et al. (2013) aqueous extract of *Hibiscus* flower have high phenolic concentration (5436.23 ± 168.6 mg GAE $100g^{-1}$). The chokeberry fruit are rich source of poliphenols compounds. The range of total polyphenols in the four cultivars of chokeberry fruit is 1845.00 ÷ 2340.00 mg GAE $100g^{-1}$ (Ochmian et al. 2012). Mean TPC in apple (*Malus pumila*) is 126.20 mg GAE $100g^{-1}$ FW (Srivastava et al. 2013). The result of Leja et al. (2007) confirm a higher concentration of total phenols in the rosehip (*Rosa canina* L.) and elderberry fruits (*Sambucus nigra* L.) compared with HF (*Crataegus monogyna* Jacq.). In fresh HF of *Crataegus monogyna* total phenols was determined at level of 62.00 mg $100g^{-1}$ FW (Leja et al. 2007). *C. monogyna* fruits are characterized by about 2.0 times higher concentration of total phenolics than yellow fruits of *C. azarolus* and about 5.0 times higher concentration of total flavonols (Mraihi et al. 2013, Mraihi et al. 2015). Total amount of total phenolics in peel of *C. monogyna* fruits collected in Tunisia is between 45.70 ÷ 123.35 mg GAE $100g^{-1}$ DW (Mraihi et al. 2013). Pliszka et al. (2016) obtained higher concentration of TPC with use of exraction with citric acid were (913.0 ± 41.8 mg GAE $100^{-1}$ FW) compared with extraction with 80% methanol (602.7 ± 17.0 mg GAE $100g^{-1}$ FW) for *C. monogyna* fruits.

In our results, aqueous extracts prepared from dry HI were richer source of total flavonoids content (TFC) than aqueous extracts from dry HF. Mean TFC in aqueous extracts from HI was nearly 4.0 times higher compared with aqueous extracts from HF. The TFC in aqueous extracts from HF and HI was analyzed in the wide range of 9.56 ÷ 457.18 and 909.26 ÷ 1795.83 mg CAE $100g^{-1}$ DW, respectively. Liu et al. (2011) described that the TFC in Chinese HF (0.2 – 1.0 mg $g^{-1}$ DM) was diminished than or close to the content in the *C. grayana* (1.0 mg $g^{-1}$ DM). Mraihi et al. (2013) obtained a higher TFC (160.35 ± 0.10 and 60.45 ± 0.06 mg rutin $100g^{-1}$ DW, respectively) compared to TPC (122.26 ± 0.16 and 60.89 ± 0.04 mg GAE $100g^{-1}$ DW, respectively) for pulp of *C. monogyna* and *C. azarolus*. A higher TFC (198.53 ± 0.11 mg eq. rutin $100g^{-1}$ DW) was analyzed in the red variety of *C. monogyna* than in yellow variety of *C. azarolus* (155.40 ± 0.23 mg rutin $100g^{-1}$ DW) in the peel of HF (Mraihi et al. 2013). Especially significant for the TPC and TFC in aqueous extracts from HF is time when fruits has been harvested. In the Barros et al. (2010) research, authors proved that unriped HF (*C. monogyna*) are the richest in phenolics and flavonoids compounds which significantly affects antioxidant properties (Barros et al. 2010). In our results the wide range of TPC may also be caused by the storage of fruits. Research of Liu et al. (2018 b) suggests that during storage of the hawthorn wine, the TPC gradually decreased. After 180 days of storage of hawthorn wine, the TPC decreased from 1.8 to 1.1 mg $mL^{-1}$.

In Poland, the estimated consumption of total polyphenols intake is 989.3 mg $day^{-1}$ (Witkowska et al. 2015). Daily intake of total polyphenols compounds in the daily diet increases in contribiuton to the consumption of two glasses of dietary supplements and herbal blends containing dried hawthorn fruit a day. The consumption of studied here aqueous infusions from HF and HI covers the estimated daily intake of total polyphenols in approximately 4.7% (46.58 mg $day^{-1}$) and 15.12% (149.56 mg $day^{-1}$), respectively.

Antioxidative effects. Referring to the results, aqueous extract prepared from HI contained higher radical scavenging activity compared to HF (Table 2). The percentage of inhibition of DPPH radicals ($I_{DPPH}$%) of aqueous extracts from HF and HI was found in the range of 11.48 ÷ 94.95% and 92.37 ÷ 93.08% respectively. Alike, the inhibition percentage of ABTS radicals ($I_{ABTS}$%) showed higher antiradical activity of aqueous extracts from HI (55.61%) compared with HF (17.45%). Pliszka et al. (2016) obtained slightly higher results in fresh hawthorn fruits of *Crataegus monogyna* from the Experimental Garden of the University of Warmia and Mazury in Olsztyn (Poland). In this study the $I_{DPPH}$% and $I_{ABTS}$% was about 85% and 55% respectively for citric acid extract of HF. The results of Ishiwata et al. (2004) also shows that the radical scavenging activity of dried fruits was diminished than corresponding fresh fruits.

**Table 2. Antioxidant activities of aqueous extracts of dry and commercial HF and HI as determined using DPPH and ABTS method.**

| Sample | DPPH *** | | | | ABTS *** | | |
|---|---|---|---|---|---|---|---|
| | I [%] | [mg TE/100g DW] | [mM TE/100g DW] | $IC_{50}$ [mg/mL] | I [%] | [mg TE/100g DW] | [mM TE/100g DW] |
| Hawthorn fruits (HF) | | | | | | | |
| FG1 | 94.17 ± 0.06 | 414.68 ± 0.11 | 1.66 ± 0.01 | 0.93 ± 0.01 $^{a}$ | 23.53 ± 1.31 $^{a}$ | 106.09 ± 7.58 $^{a}$ | 0.42 ± 0.01 $^{a}$ |
| FG2 | 89.79 ± 0.06 $^{a}$ | 390.10 ± 0.19 $^{a}$ | 1.56 ± 0.02 $^{a}$ | 1.03 ± 0.05 $^{b}$ | 24.47 ± 1.08 $^{ab}$ | 107.84 ± 5.61 $^{ab}$ | 0.43 ± 0.01 |
| FG3 | 92.00 ± 0.12 $^{b}$ | 403.06 ± 0.44 $^{b}$ | 1.61 ± 0.01 $^{b}$ | 0.53 ± 0.01 | 44.77 ± 1.29 | 215.52 ± 4.08 | 0.86 ± 0.02 $^{b}$ |
| FG4 | 89.49 ± 0.12 $^{c}$ | 387.48 ± 0.80 $^{c}$ | 1.55 ± 0.03 | 0.86 ± 0.04 $^{c}$ | 22.33 ± 1.53 $^{abc}$ | 99.38 ± 7.64 $^{abc}$ | 0.40 ± 0.01 |
| FG5 | 94.95 ± 0.06 | 418.96 ± 0.20 $^{d}$ | 1.67 ± 0.01 $^{c}$ | 2.65 ± 0.20 | 20.13 ± 0.87 $^{cd}$ | 85.83 ± 4.24 $^{d}$ | 0.34 ± 0.01 |
| FG6 * | 95.46 ± 0.06 | 420.81 ± 1.74 $^{d}$ | 1.68 ± 0.02 $^{c}$ | 4.84 ± 0.20 | 5.90 ± 0.66 $^{e}$ | 17.55 ± 3.35 $^{e}$ | 0.07 ± 0.01 |
| FG14 | 11.73 ± 0.01 | 51.53 ± 0.12 | 0.21 ± 0.02 | 22.88 ± 0.30 | 5.13 ± 0.80 $^{e}$ | 12.27 ± 3.68 $^{eg}$ | 0.05 ± 0.01 |
| FG15 | 11.48 ± 0.01 | 50.12 ± 0.14 | 0.20 ± 0.01 | 21.92 ± 0.20 | 3.03 ± 0.15 | 2.73 ± 0.73 $^{e}$ | 0.02 ± 0.01 |
| FG7 ** | 89.76 ± 0.06 $^{ac}$ | 388.12 ± 2.05 $^{ac}$ | 1.56 ± 0.01 $^{a}$ | 1.21 ± 0.01 | 22.17 ± 2.43 $^{abcd}$ | 97.58 ± 12.05 $^{abcd}$ | 0.40 ± 0.02 $^{a}$ |
| FG13 ** | 84.88 ± 0.12 | 364.49 ± 2.88 | 1.46 ± 0.01 | 0.44 ± 0.01 | 3.03 ± 0.29 | 2.95 ± 1.49 $^{eg}$ | 0.02 ± 0.01 |
| Mean | 75.37 | 328.94 | 1.32 | 5.73 | 17.45 | 74.77 | 0.30 |
| Hawthorn inflorescence (HI) | | | | | | | |
| IG8 | 93.08 ± 0.05 $^{d}$ | 406.89 ± 1.23 $^{e}$ | 1.63 ± 0.02 $^{d}$ | 0.52 ± 0.01 $^{b}$ | 41.50 ± 1.35 $^{f}$ | 191.43 ± 6.63 $^{f}$ | 0.76 ± 0.02 $^{c}$ |
| IG9 | 92.95 ± 0.06 $^{de}$ | 407.27 ± 1.44 $^{ef}$ | 1.63 ± 0.01 $^{de}$ | 0.62 ± 0.01 | 36.87 ± 2.71 $^{f}$ | 175.53 ± 13.81 $^{f}$ | 0.70 ± 0.01 $^{cd}$ |
| IG10 | 92.37 ± 0.10 $^{b}$ | 404.59 ± 1.25 $^{befg}$ | 1.62 ± 0.01 $^{bdef}$ | 0.36 ± 0.04 $^{d}$ | 66.37 ± 1.60 | 319.57 ± 8.02 | 1.28 ± 0.02 $^{bcde}$ |
| IG11 | 92.98 ± 0.10 $^{def}$ | 406.38 ± 2.82 $^{befg}$ | 1.62 ± 0.01 $^{befg}$ | 0.30 ± 0.02 $^{d}$ | 79.67 ± 0.71 | 389.93 ± 3.58 | 1.56 ± 0.02 $^{bcdef}$ |
| IG12 | 92.98 ± 0.08 $^{def}$ | 407.02 ±1.28 $^{efg}$ | 1.63 ± 0.01 $^{dfg}$ | 0.84 ± 0.03 $^{ac}$ | 53.63 ± 0.47 | 255.89 ± 2.36 | 1.02 ± 0.02 $^{cdef}$ |
| Mean | 92.87 | 406.43 | 1.63 | 0.53 | 55.61 | 266.47 | 1.06 |

* products with an ecological certificate, ** FG7 – hawthorn fruit (40 %), apple fruit, chokeberry fruit, hibiscus flower, citric acid and FG13 – hawthorn fruit (51 %), hibiscus flower, hawthorn inflorescence (10 %), rosehip, *** - TE – Trolox, DW – dry weight. The small letters indicate the no statistically significant differences ($p > 0.05$) between samples tested.

In our results, mean values of the antioxidative activities of DPPH radicals for HF and HI was 1.32 mM TE $100g^{-1}$ DW and 1.63 mM TE $100g^{-1}$ DW, respectively. Similarly results was shown by Froehlicher et al. (2009). In this study, for extracts of *C. monogyna* the decreasing order efficiencies in the DPPH system as follows: red cell > flowering tops > flowers > dry fruits > fresh fruits > yellow cell suspension. The higher values of ABTS and DPPH were calculated for dry flowers and dry flowering tops compared to dried fruits or fresh fruits (Froehlicher et al. 2009). After the tests, it was also found that the antioxidant activity of aqueous extract of dry HF and HI was more significant for HI with lowest $IC_{50}$ values (mean values 0.53 mg $mL^{-1}$). An aqueous extracts prepared from uncrushed dry HF (FG14 and FG15) revealed very low antioxidant activity with high $IC_{50}$ values – 22.88 ± 0.32 mg $mL^{-1}$ and 21.92 ± 0.25 mg $mL^{-1}$, respectively. In our results, aqueous extracts prepared from uncrushed dry HF (FG14 and FG15) characterized by the lowest radical scavenging activity from all of the samples of HF. Fragmentation degree of medicinal plant raw material and high temperature extraction conditions can affect the degradation of thermolabile compounds, the formation of pro-oxidants and complex compounds, which may contribute to the reduction of antioxidant content and antioxidant potential. On the other hand, high temperature of extraction can lead to the disruption of the cell structure, which may result an increased bioavailability of compounds present in it. (Bąk-Sypień et al. 2017).

Ascorbic acid (ASAC), organic acids content. Similarly to the previous our results, aqueous extracts prepared from HI characterized by the higher content of ascorbic acid, malic, oxalic and tartaric acid (Table 3). The mean content of ascorbic acid in aqueous extracts from HI is slightly higher (29.32 mg $100g^{-1}$ DW) compared with extracts from HF (22.02 mg $100g^{-1}$ DW). Pereira et al. (2013) obtained similar results. In this study ascorbic acid was not found in *C. monogyna* fruits, but in flowers and flowering shoots ascorbic acid was analyzed at the level of 2.14 ± 0.21 mg $g^{-1}$ DW. Barros et al. (2010) analyzed ascorbic acid in flowers of *Crataegus* at the mean level of 408.37 mg $100g^{-1}$ DW and 130.33 mg $100g^{-1}$ DW in unripe fruits, 220.24 mg $100g^{-1}$ DW in ripened fruits and only 28.40 mg $100g^{-1}$ DW in over ripened fruits of *C. monogyna*. It should be noted, that the drying method used for the production of herbal blends and high-temperature extraction used in our studies (100°C) can decrease content of ascorbic acid in aqueous extract of HF and HI (Przybylska et al. 2018). In turn, Ishiwata et al. (2004) claims that contribution of ascorbic acid in dried HF purchased from markets in Japan is negligible and suggests that the radical scavenging activity may originate from other active compounds, such as polyphenols.

**Table 3. Content of ascorbic acid and organic acids in the aqueous extracts of dry HF and HI.**

| Sample | Organic acid [mg/100g DW] *** | | | | | Total acidity **** [mg/100mL] |
|---|---|---|---|---|---|---|
| | ascorbic | citric | malic | oxalic | tartaric | |
| Hawberries (hawthorn fruits) (HF) | | | | | | |
| FG1 | 25.20 ± 3.35 $^{A}$ | 50.77 ± 1.15 | 381.24 ± 7.75 $^{a}$ | 756.02 ± 8.50 | 175.38 ± 14.50 $^{a}$ | 108.07 ± 0.25 $^{a}$ |
| FG2 | 24.37 ± 1.69 $^{B}$ | 134.97 ± 3.06 | 267.52 ± 11.38 $^{b}$ | 603.99 ± 9.87 | 414.56 ± 10.60 $^{b}$ | 105.07 ± 0.65 |
| FG3 | 27.61 ± 3.68 $^{C}$ | 118.84 ± 2.81 | 400.85 ± 14.43 $^{a}$ | 381.75 ± 9.31 $^{a}$ | 320.57 ± 36.23 $^{c}$ | 102.87 ± 0.75 $^{b}$ |
| FG4 | 29.90 ± 3.70 $^{D}$ | 57.92 ± 3.20 $^{a}$ | 141.41 ± 16.97 $^{c}$ | 1217.23 ± 3.08 | 90.07 ± 4.69 | 108.60 ± 0.56 $^{a}$ |
| FG5 | 29.31 ± 1.81 $^{BE}$ | 58.72 ± 2.10 $^{a}$ | 202.81 ± 8.95 $^{d}$ | 379.24 ± 9.47 $^{ab}$ | 227.64 ± 3.08 $^{d}$ | 102.30 ± 0.20 $^{bc}$ |
| FG6 * | 36.79 ± 3.75 $^{ABCEF}$ | 86.11 ± 3.18 | 113.53 ± 8.23 $^{c}$ | 332.00 ± 9.89 | 39.72 ± 5.05 | 101.47 ± 0.61 $^{bcd}$ |
| FG14 | 23.80 ± 2.80 $^{DEFH}$ | 1917.62 ± 1.90 | 190.66 ± 2.39 $^{d}$ | 70.91 ± 9.45 | 611.92 ± 2.11 $^{e}$ | 19.03 ± 0.21 |
| FG15 | 23.25 ± 2.71 $^{DEFH}$ | 1990.65 ± 4.10 | 204.42 ± 2.73 $^{d}$ | 178.30 ±14.71 | 293.57 ± 4.41 $^{cf}$ | 42.93 ± 0.35 |
| FG7 ** | nd | 844.15 ± 1.20 | 268.42 ± 9.71 $^{be}$ | nd | 50.62 ± 1.75 | 124.67 ± 0.65 |
| FG13 ** | nd | 3355.10 ± 4.44 | 273.24 ± 12.38 $^{be}$ | nd | 1204.58 ± 1.02 | 138.97 ± 0.25 |
| Mean | 22.02 | 861.48 | 244.41 | 391.94 | 342.86 | 95.40 |
| Hawthorn inflorescence (HI) | | | | | | |
| IG8 | 26.44 ± 3.10 $^{FG}$ | 641.90 ± 5.62 | 298.75 ± 3.86 $^{gf}$ | 1593.52 ± 4.81 | 588.19 ± 17.17 $^{e}$ | 95.17 ± 0.15 $^{e}$ |
| IG9 | 28.89 ± 3.30 | 258.33 ± 1.20 $^{a}$ | 314.08 ± 5.98 | 485.38 ± 5.22 | 252.26 ± 47.38 $^{acdf}$ | 95.17 ± 0.40 $^{ef}$ |
| IG10 | 29.97 ± 3.21 $^{BFGH}$ | 449.10 ± 3.17 | 247.06 ± 3.36 | 1053.30 ± 5.05 | 431.72 ± 35.85 $^{b}$ | 95.10 ± 0.10 $^{ef}$ |
| IG11 | 30.80 ± 3.11 $^{ABH}$ | 271.52 ± 11.60 $^{bc}$ | 276.14 ± 2.85 $^{bef}$ | 660.00 ± 25.98 | 1645.02 ± 19.53 | 89.97 ± 1.06 |
| IG12 | 30.50 ± 3.05 $^{BF}$ | 270.88 ± 2.07 $^{c}$ | 297.73 ± 0.83 $^{g}$ | 415.84 ± 25.72 $^{ab}$ | 515.75 ± 13.24 | 100.43 ± 0.31 $^{d}$ |
| Mean | 29.32 | 378.34 | 286.75 | 841.61 | 686.59 | 95.17 |

* - products with an ecological certificate, ** - FG7 – hawthorn fruit (40 %), apple fruit, chokeberry fruit, hibiscus flower, citric acid and FG13 – hawthorn fruit (51 %), hibiscus flower, hawthorn inflorescence (10 %), rosehip, *** - nd - not detected, DW – dry weight, **** - expressed as the mg of citric acid per 100 mL. The big and small letters indicate the statistically significant differences ($p < 0.05$) and no statistically significant differences ($p > 0.05$) between samples tested.

In our results the highest citric acid content analyzed for sample FG13 (3355.10 ± 4.44 mg $100g^{-1}$ DW). It should be emphasized that this value is not commensurate with the naturally occurring concentration of this acid in the HF, due to the addition different part of medicinal plants, i.e. hawthorn inflorescence, hibiscus flower and rosehip. The high citric acid content was found in aqueous extracts prepared from uncrushed dry HF in samples FG14 and FG15 (1917.62 ± 1.90 mg $100g^{-1}$ DW and 1990.65 ± 4.10 mg $100g^{-1}$ DW respectively), while the citric acid content in the extracts from crushed HF was in the range of 50.77 ÷ 134.97 mg $100g^{-1}$ DW (Table 3). The aqueous extracts from HF is richer in citric acid content than HI over 2.0 times. Pande & Akoh (2010) obtained similar results.

The whole fruits and leafs of *Crataegus sp.* characterized by citric acid content at the level 17.1 ± 3.8 mg $100g^{-1}$ FW and 8.5 ± 3.0 mg $100g^{-1}$ FW. In turn Pereira et al. (2013) described citric acid content in *C. monogyna* fruits at the level of 0.73 ± 0.03 mg $g^{-1}$ DW, while in the flowers and flowering shoots at the level 8.33 ± 0.07 mg $g^{-1}$ DW. Gundogdu et al. (2014) demonstrated that the highest citric acid content is in *C. pseudoheterophylla* fruits at level of 25.69 ± 0.04 g $100g^{-1}$ FW. In another study, the citric acid content was the richest in *C. pinnatifida* fruits compare with *C. brettschneideri* and *C. scabrifolia* (Liu et al. 2010).

The mean malic acid concentration in aqueous extracts prepared from dry HF (244.41 mg $100g^{-1}$ DW) is comparable with extracts from dry HI (286.75 mg $100g^{-1}$ DW). See Table 3 and Figure 2. In turn, Pande & Akoh (2010) described the malic acid content in whole fresh fruit at the level of 1460.00 ± 45.30 mg $100g^{-1}$ FW, while in the leafs at the level only 658.00 ± 22.40 mg $100g^{-1}$ FW. Among eleven analyzed HF species, *C. pseudoheterophylla* characterized by the highest malic acid content (2.67 ± 0.05 g $100g^{-1}$ FW) (Gundogdu et al. 2014). In *C. pinnatifida* malic acid was not found (Liu et al. 2010).

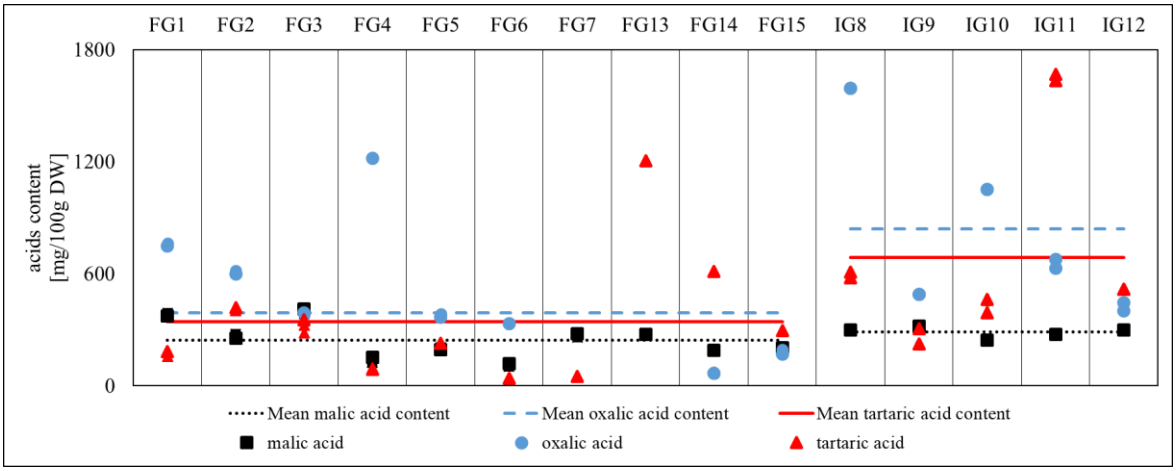


**Figure 2. Malic acid, oxalic acid and tartaric acid content in aqueous extracts obtained from dry HF (FG1 – FG15) and HI (IG8 – IG12).**

As shown in Figure 2 and Table 3, the oxalic acid content was determined at the of level 841.61 mg $100g^{-1}$ DW in aqueous extracts prepared from dietary supplements containing HI, while in the extracts from HF 391.94 mg $100g^{-1}$ DW. In the other study, oxalic acid was absent in whole HF but present in the hawthorn leafs at the level 18.6 ± 3.90 mg $100g^{-1}$ FW (Pande & Akoh 2010). In the study of Gundogdu et al. (2014), the highest oxalic acid content was measured in *C. monogyna subsp. azarella* fruits at the level of 3.28 ± 0.15 g $100g^{-1}$ FW, while the lowest in the *C. szovitsii* at the level of 0.54 ± 0.00 g $100g^{-1}$ FW. The decreased results were calculated for *C. monogyna* fruits obtained from western and central Spain. Mean oxalic acid content was at the level of 57.40 ± 26.94 mg $100g^{-1}$ FW (Morales et al. 2013). In turn higher oxalic acid concentration was found in *C. monogyna* fruits at the level of 2.10 mg $g^{-1}$ DW and *C. monogyna* flowers and flowering shoots 9.15 ± 0.88 mg $g^{-1}$ DW (Pereira et al. 2013).

The content of tartaric acid in aqueous extract of dry HI is about 2.0 times higher than extract from HF (Figure 2). According to the data, organic acid content of fruits vary depending on the species (Gundogdu et al. 2014). Chinese HF have higher organic acid content compare with variety of *Crataegus* fruits originated from Europe (Jurikova et al. 2012). According to results of Gundogdu et al. (2014) *C. pseudoheterophylla* fruits had the highest tartaric acid content (2.22 ± 0.04 g $100g^{-1}$ FW), while *C. atrosanguinea* had the diminished tartaric acid content (0.61 ± 0.03 g $100g^{-1}$ FW). Edwards et al. (2012) described content of tartaric acid in cultivars of *C. scabrifolia*, *C. sanguinea*, *C. cuneata* and *C. pinnatifida* at the level of 21.9, 16.9, 11.2 and 16.3 mg $g^{-1}$, respectively.

Correlation analysis for aqueous extracts prepared from dry hawthorn fruits (HF). In the present study, the positive correlation was observed between TPC and TFC ($r = 0.70$, $p < 0.05$). See Figure 3. García-Mateos et al. (2013) obtained the lower Pearson's correlation between phenolic and flavonoid content in HF (*Crataegus* spp.) of Mexico, commonly name tejacote ($r = 0.53$, $p < 0.05$).

Results showed that TPC, TFC is positively and highly correlated with fragmentation degree expressed as bulk density ($r = 0.73$, $p < 0.05$ and $r = 0.79$, $p < 0.05$ respectively). Dmowski et al. (2014) did not found any statistical significance between content of total polyphenols and fragmentation degree of infusions of selected black teas regardless of the time of extraction (3 and 15 minutes).

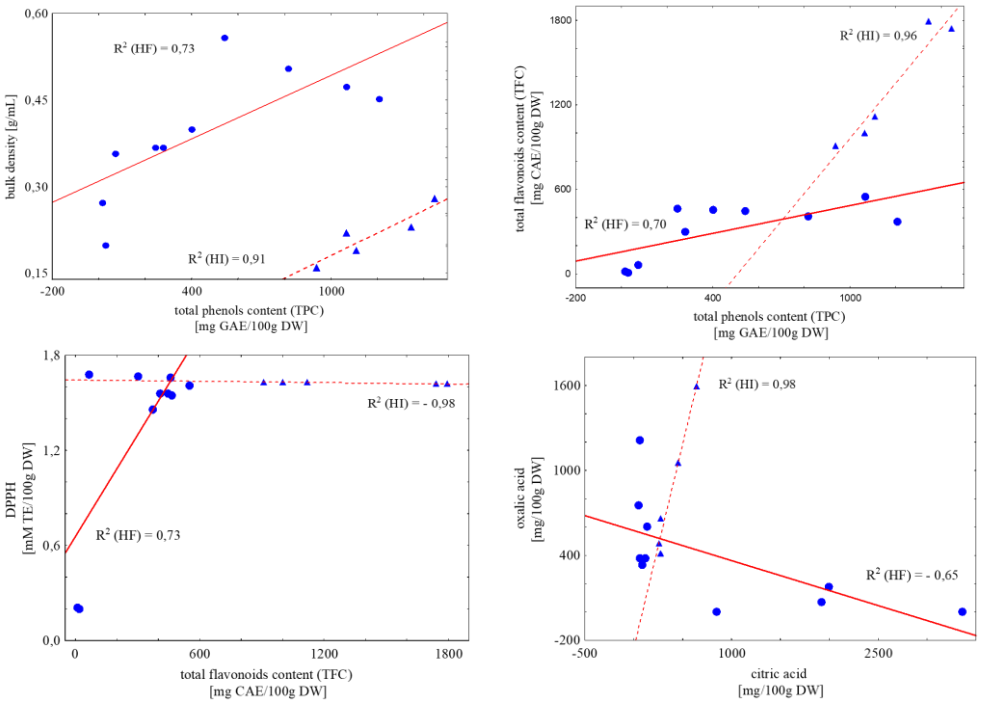


**Figure 3. The relationship ($p < 0.05$) between parameters of the tested aqueous extracts prepared from dry HF (●) and HI (▲).**

In our results, no correlation was observed between TPC and antioxidative activity with DPPH and ABTS radicals in aqueous extracts prepared from dry hawthorn fruits. Similar effects was observed in Mraihi et al. (2013) study. In this study correlation between TE equivalent antioxidant capacity (TEAC) and total phenolic contents of *C. monogyna* had a coefficient varied between 0.32 ÷ 0.97 while the high correlation coefficient was calculated of *C. azarolus* was 0.99 and 0.82. In turn, high correlation was observed between TFC and DPPH ($r = 0.74$, $p < 0.05$) and ABTS values ($r = 0.85$, $p < 0.05$). No correlation between ascorbic acid content and antioxidative activities with DPPH and ABTS radicals may indicate that the ascorbic acid has no effect on radical scavenging activity (Ishiwata et al., 2004). The study of correlation relationships suggested that fragmentation degree and flavonoid compounds are responsible for antioxidant properties of aqueous extract of dietary supplements and herbal blends containing dry HF. Also, very strong correlations was found between total acidity and antioxidant capacity with DPPH assay ($r = -0.95 \div 0.90$, $p < 0.05$), while no correlation was observed between total acidity and ABTS values.

The high correlation coefficient was found between citric and oxalic content ($r = -0.65$, $p < 0.05$), while correlation between citric and tartaric acid was $r = 0.82$ ($p < 0.05$). A slightly decreased correlation coefficient was calculated between citric and tartaric acid for pomegranate ($r = 0.68$, $p < 0.01$) (Fawole & Opara 2013).

Correlation analysis for aqueous extracts prepared from dry hawthorn inflorescences (HI). The analysis of Pearson's correlation coefficients of HF showed the very strong correlation between TPC and TFC ($r = 0.96$, $p < 0.05$) in aqueous extracts prepared from dry hawthorn inflorescences (Figure 3). Also, the very high correlation coefficients was measured between TPC and fragmentation degree of dietary supplements and herbal blends containing only HI ($r = 0.91$, $p < 0.05$). The high negative correlations was observed between TPC, TFC and antioxidative activities with DPPH ($r = -0,94$ and $r = -0,98$, $p < 0.05$), while strong positive correlation between TPC, TFC and antioxidative activities with ABTS ($r = 0,80$ and $r = 0,83$, $p > 0.05$). There were also very strong and negative correlation between malic acid content and TFC ($r = -0.89$, $p < 0.05$), DPPH value ($r = 0.97$, $p < 0.05$). Similarly, the significant positive high correlation was observed between oxalic acid and citric acid content in aqueous extracts from dry HI ($r = 0.98$, $p < 0.05$).

Principal component analysis (PCA). The first two principal components (P1 and P2) explained 82.26% of the variance of collected data (Figure 4A). The results of PCA showed that two distinct groups were separated with line. The first two principal components performs almost a perfect separation of dietary supplements and herbal blends containing HF and HI with exception of the FG3 sample characterized by very high TPC (1066.97 ± 24.48 mg GAE $100g^{-1}$ DW), TFC (548.41 ± 0.40 mg CAE $100g^{-1}$ DW) and the highest malic acid content (400.85 ± 14.43 mg $100g^{-1}$ DW). These features may explain the proximity of the FG13 sample to HI on the PCA plane. The high concentration of TPC, TFC and malic acid in FG3 may indicate that the unripe fruit of HF were harvested. Unripe *C. monogyna* fruits characterized by the highest phenolics content and flavonoids content compare with ripened and over ripened fruits (Barros et al. 2010, Bahorun et al. 2003). Similarly properties was shown by Fawole & Opara (2013) analyzing polyphenols, flavonoids and malic acid contents at five different maturity stages of pomegranate. The position of the sample FG13 is probably conditioned the highest concentration of citric acid (3355.10 ± 4.44 mg $100g^{-1}$ DW) and TPC (1206.79 ± 18.30 mg GAE $100g^{-1}$ DW) in all analyzed samples. This properties can be related by the addition of parts of different medicinal plants to herbal blends (51% HF, hibiscus flower, 10% HI and rosehip) by the manufacturer. The similarity of samples belonging to group I (FG5, FG6, FG14 and FG15) characterized by the least fragmented HF and the diminished TPC, TFC, malic and oxalic acids contents, compared to the other groups.

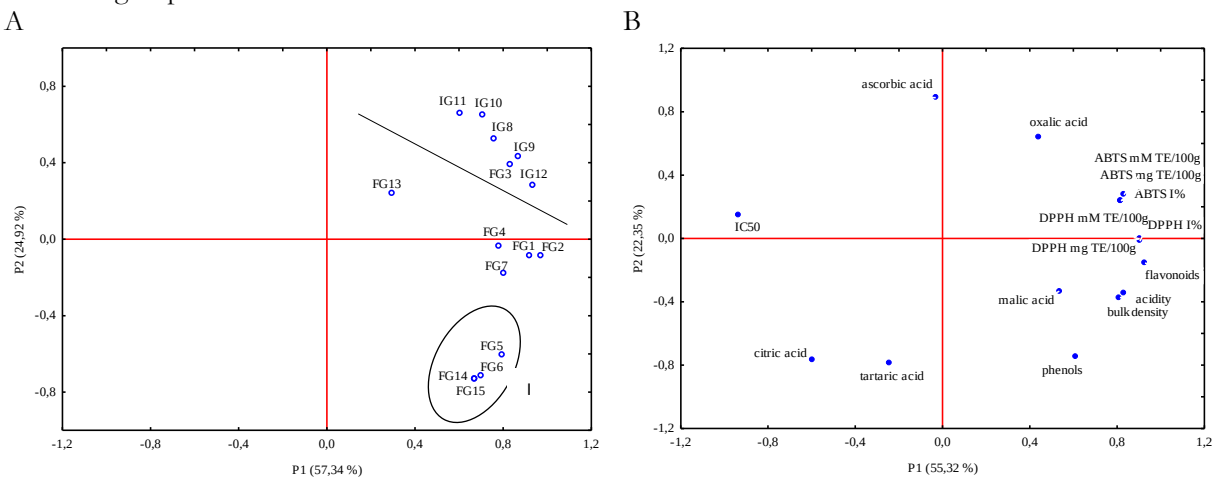


**Figure 4. Principal component analysis (PCA) of the first two principal components (P1 and P2). PCA biplot of the TPC, TFC, antioxidative effects with DPPH and ABTS radicals, organic acids contents and total acidity of aqueous extracts of dietary supplements and herbal blends containing HF and HI (A) and samples containing only HF (B).**

In the Figure 4B, the first two principal components (P1 and P2) explained 77.67% of the variance of data. The PCA showed that TFC have short distance to the antioxidant activity in DPPH assay, what suggest the significant contribution these compounds to the antioxidant capacity aqueous extracts of dry HF. The high correlation between TFC and DPPH assay confirms this data (Figure 3).

Ruiz-Rodrigez et al. (2014) used PCA to classify the wild *C. monogyna* fruits according to their active compounds. Significant strong correlation were found between TPC with antioxidant capacity measured by FRAP and ABTS assay. Authors indicated that phenolic compounds are the main contributor to the antioxidant capacity of HF and blacktorn (Ruiz-Rodrigez et al. 2014). In our study, bulk density and total acidity lie close to each other indicating a positive strong correlation ($r = 0.81$, $p < 0.05$) between this two trait in dry HF. Similarly, bulk density and DPPH assay lie close to each other ($r = 0.75$, $p < 0.05$), while $IC_{50}$ value lies on the opposite diagonal corner ($r = - 0.82$, $p < 0.05$).

## Conclusions

The results of this study demonstrated clear differences between biochemical composition and antioxidant activity of aqueous extracts of dietary supplements and herbal blends containing dry hawthorn fruits and hawthorn inflorescences. The total phenols and flavonoids content increased with the increasing degree of dry hawthorn fruits fragmentation. The present study shows that the addition the parts of other medicinal plant to herbal blends may contribute to the increase of total phenols content in aqueous extract of dry hawthorn fruits. An aqueous extracts containing dry hawthorn inflorescences are a richer source of active compounds compare with extracts with dry hawthorn fruits. We can speculate that dry hawthorn inflorescences have higher health prevention potential compare with hawthorn fruits. The principal component analysis (PCA) shows clear differences between the chemical composition of aqueous extracts of hawthorn fruits and hawthorn inflorescences.

## Acknowledgement

The support from the Nicolaus Copernicus University, Collegium Medicum (Bydgoszcz, Poland) by internal grant UPB-407 and UPB-449 is kindly acknowledged.

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