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Evalation of Antioxidant and Antimicrobial Activities of *Crataegus monogyna*

PUGNA Andreea-Adelina, Imola-Noemi NAGY, Sonia-Ancuta SOCACI, Bogdan HODIŞAN, Suzana-Elena BIRIŞ-DORHOI*, Maria TOFANĂ
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
Faculty of Agriculture, Department of Crop Science,
Technology Transfer Centre BIOTECH,
3-5 Calea Mănăştur, 400372, Cluj-Napoca, Romania
*Correspondence: elena.biris@usamvcluj.ro

Abstract: Antioxidant capacity and antimicrobial activity are tremenously importance process that possess the ability to protect the body from damage and infection caused by free radicals and bacteria. The bioactive compounds of *Crataegus monogyna* were evaluated in order to valorize these products as sources of natural additive. Analytical techniques such as extraction, determination of total polyphenols and flavonoids, demonstrative antioxidant and antimicrobial of extracts from *C. monogyna* were done in order to evaluate conservation capacity. Polyphenols are the major source of bioactive compounds obtained from the small red fruits of the hawthorn plant. Hawthorn fruit extracts could inhibit the oxidation of low-density lipoprotein. Due to increased interest in fruit consumption and growing of it products, there is positive effect on fruit polyphenols on human healt.

Keywords: antimicrobial activity, antioxidant capacity, *Crataegus monogyna*, flavonoids, polyphenols.

Introduction

Crataegus monogyna, (Hawthorn) genus, is represented by nearly 200 species in the world. This herb falls under the largest genus *Crataegus*, belongs to the *Rosaceae* family and is endemic in the temperate regions such as Europe, Asia and North Africa.

C. monogyna, grow as bushes and trees as small 10m. Their flowers 8-15 mm in diameter are white or pink. The leaves are prickly and long, ovate or obovate. Their fruits 6-10mm in diameter and hairless are red or brownis-red color, and spherical or oval shape (Wyk, 2005).

Crataegus, species are know for their useful biological effects for many years. The use of *Crataegus,* species in the treatment of cardiovascular diseases dates back to the 1800s. Previous studies made on heart and blood preassure disorders, diarhea, biliary cirrhosis, insomnia and asthma are also know. Hawthorn extracts had the ability to reduce the inclusiveness of inflammation hypertension and thrombosis (Bernatoniene et al.,2008).

There are monographs of leaves, flowers, and fruits of *C. monogyna,* in the European Pharmacopeia. In the ESCOP monographs, the leaves and flowers of the plant are registered to reduce cardiac performance (Bruneton, 2009). Althought clinical trials generate contradictory results, ethanolic extracts of moderate forms are typically used nowadays as positive inotropic, anti-arrhythmic and vasodilator agents (Christaki et al., 2021).

Medicinal plants and herbs was of incredible significance in the remedy of diseases since ancient times. In the past two decades, there was a drastic boom in the usage of herbal medicines as natural remedies for human aliments (Benkirane, 2018).

The purpose of this study is to establish the bioactive compounds while revealing their antioxidant and antimicrobial activities, evaluated in order to valorize these products as sources of natural food additives.

Materials and methods

Material

Aerial parts (fruits, leaves and flowers) or frish plant *Crataegus monogyna,* were harvested from the wild all six several locations of Bistrița-Năsăud and Cluj-Napoca, in May and June 2022 (leaves and flowers) and in Septembre and October (fruits).

Plants were kept in cooled bag for transport to the laboratory. The seamples were cleaned in an air screen cleaner to remove all foreign matter such as dust, dirt, immature and damaged fruits. The initial moisture content of fruits was determined by using a standard method.

Plant parts of *C.monogyna,* contain various bioactive compound contributing to the bioactivity. Previously different plant parts of C. *monogyna* were studied contain a variety of bioflavonoid like complex that appear to be primarily include oligomeric procyanidins, vitexin, quercetin and hyperoside. Other chemical constituents includes vitamin C, saponins, tanins, isobutylamine, purine and ursolic acid (Benkirane, 2018; Hakima et al., 2019).

Analytical techniques

Extraction is a predominat step in the analytical processing of plants. Both conventional and non-conventional methods have been applied for the extraction of bioactive compounds from the *C. monogyna* plant (Dai and Munper, 2010). Conventional methods such as Soxhlet and maceration and non-conventional methods such as supercritical fluid extraction (SFE), microwave-assisted extraction (UAE) have been applied for the extraction of bioactive compounds (Proestos, 2008; Ravikumar and Chua, 2022).

Plant Extracts

Extraction was carried out by maceration in methanol and extracts were evaporated at 35°C under reduce pressure, dissolved in methanol at 10mg/ml and stored at 4°C for subsequent use in calorimetric assy and antioxidant activity (Ong et al., 2021; Babiker et al.,2020).

Polyphenols

Determination of total polyphenols was carried out according to (Li et al., 2007) using Folin Ciocalteu reagent.

75 ml water, 10g of sodium, 2g of phosphomolybdic acid and 15ml of phosphoric acid were mixed and boiled for 2h. After cooling the mixture was completed to 100ml with deionised water, 200μl of each hawthorn extract solution was mixed with 1ml of Folin Ciocalteu solution diluted ten-fold. After 4 min, 800μl of sodium carbonate was added to the solution and incubated for 2h in the dark.

The absorbance was determined at 765nm using the spectrophotometer.Gallic acid solutions ranging from 0 to 200μg/ml were prepared and used to establish a standard calibration curve (Nabavi, 2015).

Flavonoids

Aluminium chloride method was used to quantifi flavonoids according to the method used by (Chinedu et al., 2017). Hawthorn extract solution, ranging from 0 to 35 μg/ml were prepared and mixed with $AlCl_3$ (2% in methanol). After 10 min., the absorbance was measured at 430nm. A calibration curve was established with quercetin (Chinedu et al., 2017).

Antioxidant capacity

Antioxidant power of methanolic extracts of flowers, leaves and fruits of *C. monogyna* againist DPPH radical was evaluated by spectrophotometry using the method describe by (Righi et al., 2021). A series of dilutions were performed in order to obtain concentrations ranging from 5µg/ml to 0.4mg/l for hawthorn extracts and from 1 µg/ml to 0.4 mg/ml for ascorbic acid. A methanolic solution of DPPH 0.8m was prepared and 0.25ml, of this solution was added to 3.75 µl of extract solutions (Righi et al., 2017; Sanchez et al., 2017).

The resulting mixtures were kept in the dark at room temperature for 30min.

The absorbance was measured at 517 nm.The results were compared with the negative control. Ascorbic acid was used as a reference antioxidant (Brewer, 2011; Shah et al., 2014).

Antimicrobial activity

The qualitative and quantitative antibacterial assay of extracts was carried out by the disc diffusion method.

500µl of the inoculums were spread over plates containing sterile Muller-Hinton agar (Taylor, 2013).

Four bacterial strains is used *Pseudomonas aeruginosa* ATCC 27852, *Escherichia coli* ATCC 25992, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* ATCC 43300. Strain were cultivated in nutrient broth and incubated at 37°C for 24h. Then, dilution was prepared in sterile physiological water for each culture and Muller Hinton agar plates were inoculated and incubated for 15 min. at 37°C. Wells 4mm in diameter were out in the agar and filled with 100µl of extract solution.

As a control 100 µl of quercetin and ascorbic acid were poured into a well in the center of each 118 Petri dish. The diameter of the inhibation zone was measured after 24h of incubation at 37°C and the activity determined as reported (Sana et al., 2013; Pluta et al., 2019; Oniga et al., 2016)

Results and discussion

Total polyphenols

Total polyphenols content was derived using the Gallic acid calibration curve and the absorbance value of each test extract.

The results showed that *C.monogyna* is rich in polyphenols with significant differences among plant parts (Table 1).

Immature fruits contained the highest concentration (284.06 µg GAE/mg extract) followed by flowers (253.35 µg GAE/mg extract), then leaves (199.50 µg GAE/mg extract), while ripened fruits registred the lowest value (105.10 µg GAE/mg extract).

Table 1

Polyphenols contents of *Crataegus Monogyna* plant part

Parametre	*Extraction yield 9/100 g dry weight*	*Polyphenols content µg EqGA/mg extract*
Flower	24.68 ± 1.81 B	253.35 ± 10.83
Leaf	13.79 ± 1.28 C	199.50 ± 05.58
Ripened fruit	34.31 ± 0.91 A	105.10 ± 03.01
Immature fruit	10.74 ± 1.01 C	284.06 ± 03. 14

Values in the same line with different lower-case letters ar significantly different at mean ± SD; n=3 and ≤0.05

Total flavonoids

Total flavonoids content derived using the quercetin calibration curve and the absorbance value of each test extract, showed significant differences among plant part (Table 2).

Flowers and leaves presented the highest values (41.28 and 39.08 µg QE/mg extract respectively), followed by immature fruits (15.79µg QE/mg extract).

Mature fruits presented the lowest value (4.69 µg QE/mg extract).

Fruit content flavonoid such as chlorogenic acid, hyperoside, rutin, quercetin, epicatechin and cyanidins.

Table 2

Flavonoids contents of *Crataegus Monogyna* plant part

Parametre	*Extraction yield 9/100 g dry weight*	*Flavonoids content µg EqGA/mg extract*
Flower	24.68 ± 1.81 B	41.28 ± 2.43
Leaf	13.79 ± 1.28 C	39.08 ± 0.82
Ripened fruit	34.31 ± 0.91 A	05.12 ± 0.23
Immature fruit	10.74 ± 1.01 C	15.79 ± 0.51

Values in the same line with different lower-case letters ar significantly different at mean ± SD; n=3 and ≤0.05

Antioxidant capacity

The principle of analyzing antioxidant activity is based on the color change of DPPH.

The values obtained are defined as the inhibitory extract concentration necessary to decrese by 50% the initial concentration of DPPH and are expressed in µg/ml. The results showed significant differences among different plant parts whit values ranging from 7.27 to 23.76 µg/ml against 2.83 µg/ml for ascorbic acid (Table 3).

Polyphenol, of different plant parts presented a strong and significant correlation with their coresponding antioxidant activity (r=0.97), whil flavonoid contient was moderately correlated (r=0.49).

This clearly showes that antioxidant potential of hawthorn extracts is mainly associated with polyphenols content.

Table 3

Flavonoids contents of *Crataegus Monogyna* plant part

Parametre	*Extraction yield 9/100 g dry weight*	*Antioxidant activity (IC-50)µg/ml*
Flower	24.68 ± 1.81 B	07.13 ± 0.83
Leaf	13.79 ± 1.28 C	11.44 ± 1.83
Ripened fruit	34.31 ± 0.91 A	18.21 ± 1.83
Immature fruit *Ascorbic acid*	10.74 ± 1.01 C	08.42 ± 0.66 03.03 ± 0.55

Values in the same line with different lower-case letters ar significantly different at mean ± SD; n=3 and ≤0.05

% inhibition = (Abs Control – Abs Test) /Abs Control ∗ 100

Antimicrobial acitivity

Antimicrobial activity of the plant extracts was evaluated four bacterial test species know to causes humans infections. Extracts of fruits and leaves were the most effective (Table 4).

The largest inhibition zone was observed with flavonols of the inner layer of fruits (40mm) inhibiting the gram negative *E.coli*. Flavonols extracts from leaves were slightly effective against *E.coli* with an inhibition zone of (12mm), (Table 4). Extracts of fruits (flavonols and anthocyanns) were also effective (30mm) but only inhibiteh the growth of *P. aeruginosa*.

None of extracts from fruits and leaves inhibited *S.aureus*.

Table 4

Diameters of inhibition zones (mm)

Bacteria species	*Fruits Inner layers*	*Leaves Inner layers*
E.coli ATCC 25992	40	13
P.aeruginosa ATCC 27852	30	20
S.aureus ATCC 25923	-	-

Conclusions

- As a conclusion the present study shows that the studied species are an extremely rich source in bioactive compounds such as polyphenols and flavonoids.
- The results also indicate high antioxidant capacity especially immature fruits and flowers.
- This study demonstrates the importance of the flavonoid content in antimicrobial activity. The flavonoid extracts from fruit and flowers showed a largest inhibition a gram negative bacteria.
- This could be useful for obtain a natural additives with high conservation value.

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