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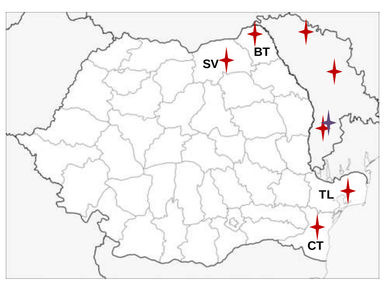
Morphological and phytochemical characterization of *C. monogyna* and *C. pentagyna* fruits from the spontaneous flora of Eastern Romania and Republic of Moldova

C.P. Stefanache[1,2*], R.M. Cretu[1], A.V. Grigoras[1], R. Necula[1,3], E. Gille[1,3]

[1] NIRDBS/ "Stejarul" Biological Research Centre, st. Al. cel Bun 6, Piatra Neamt, Romania; [2] "Al. I. Cuza" University of Iasi, Faculty of Biology, Blvd. Carol I 20A, Iasi, Romania; [3] "Al. I. Cuza" University of Iasi, Faculty of Chemistry, Blvd. Carol I 11, Iasi, Romania

** Email: camelia.stefanache@yahoo.com*

INTRODUCTION



Crataegus species are known to be used in natural health products like tinctures, tablets and teas for the treatment of heart diseases [1]. *In vitro*, the fruits, leaves and flowers of *Crataegus* species have also been shown to exhibit antioxidant and antiproliferative activities [2, 3]. However, these properties are influenced by the type of plant organ, harvesting season, species and geographical origin.

Our study aims to perform a morphological and phytochemical characterization of two *Crataegus* species, namely *C. monogyna* Jacq. (hawthorn) and *C. pentagyna* Willd. (black hawthorn), in order to identify highly productive accessions (biomass and bioactive compounds) for their prospective use in nutraceuticals and their propagation (cultivation) in order to ensure a sustainable exploitation.

In this study we focused on fruit biomass (fresh and dry weight per fruit and per 100 fruits, and drying ratio) and on several bioactive compounds (phenolics and ascorbic acid) [4, 5].

MATERIALS AND METHODS

Plant material

▪ Fruit samples were harvested in 2019 from the Eastern half of Romania (North-East and South-East areas) and from different regions of R. Moldova (North, Centre and South).

Extract preparation for phenolic compounds

▪ Dried and comminuted fruits were extracted with methanol, 3 times for 30 minutes.

Qualitative assessment of phenolic compounds by Thin Layer Chromatograpy (TLC)

▪ *Solid phase*: Kiesegel 60 F_{254} (Merck); *Mobile phase*: Ethyl acetate/Formic acid/Glacial acetic acid/Water (100:11:11:20); *Distance*: 8 cm; *Derivatization*: 2-Aminoethyldiphenyl borate (1% in methanol) (Fluka); *Detection*: 366 nm.

Quantitative assessment of phenolic compounds

▪ Assessment of total phenolic compounds, total phenolic acids and flavonoids content was performed through spectrophotometric methods [6].

Identification and quantification of phenolic compounds

▪ Identification and quantification of phenolic compounds was performed by RP-HPLC-DAD analysis [6].

Assessment of total ascorbic acid content

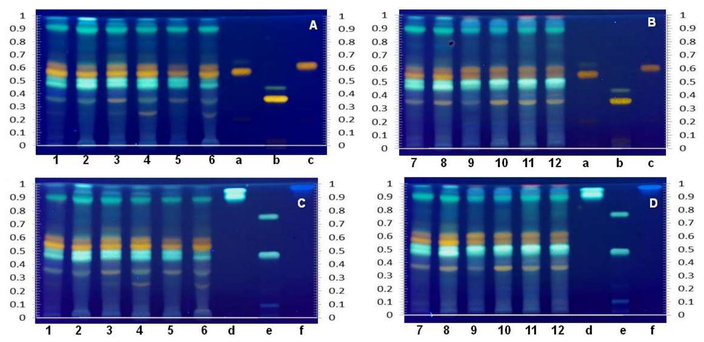
▪ Total ascorbic acid content (vitamin C) was determined by iodometric titration [7].

RESULTS AND DISCUSSIONS

Table1. *C. monogyna* and *C. pentagyna* fruit samples

Region	Harvesting site	Sample code
C. monogyna		
N-E Romania	Praxia (Pr)/SV[1]	Pr-(1); Pr-(2); Pr-(3)
	Marginea (Mg)/SV	Mg1-(1); Mg2-(1); Mg2-(2); Mg2-(3)
	Blandesti (Bl)/BT[2]	Bl-(1)
S-E Romania	Ciucurova (Cc)/TL[3]	Cc1-(3); Cc2-(3); Cc3-(1)
	Izvoarele (Iz)/TL	Iz-(1)
	Nicolae Balcescu (NB)/TL	NB-(1)
	Mircea Voda (MVd)/TL	MVd-(1)
	Mihai Viteazu (MVt)/TL	MVt1-(2); MVt2-(2)
	Caugagia (Cg)/TL	Cg-(1)
	Somova (Sm)/TL	Sm-(1)
	Cuza-Voda(CV)/CT[4]	CV1-(2-1); CV2-(2-2); CV3-(2-2)
	Cheile Dobrogei (CD)/CT	CD1-(3-2)
R. Moldova	Soroca (Sr)/North	Sr-(2)
	Orhei (Oh)/Centre	Oh-(2)
	Cociulia (Cl)/South	Cl-(2)
C. pentagyna		
R. Moldova	Cociulia (Cl)/South	Cl-(1); Cl-(2); Cl-(3); Cl-(4)

1. Suceava County, 2. Botosani County, 3. Tulcea County, 4. Constanta County

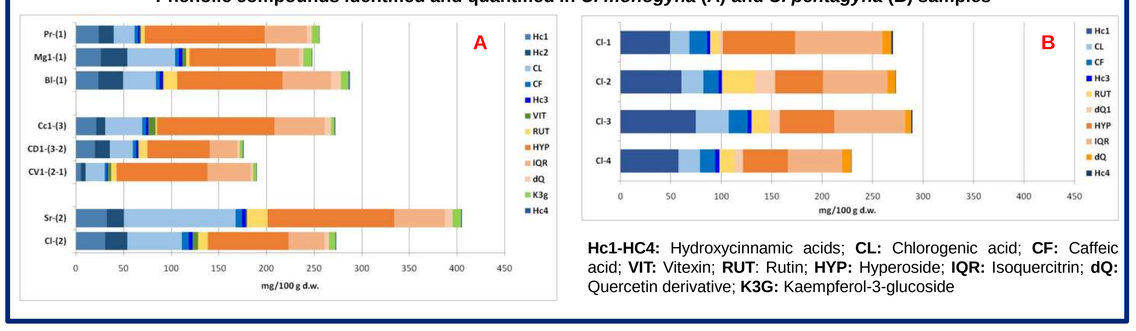


TLC Chromatograms of *Crataegus* fruit extracts

Samples: 1-8 *C. monogyna* (Pr-(1), Mg1-(1), Bl-(1), Cc1-(3), CD1-(3-2), CV1-(2-1), Cl-(2), Sr-(2)); **9-12** *C. pentagyna* (Cl-(1), Cl-(2), Cl-(3), Cl-(4)). **Reference substances: a.** Hyperoside; **b.** Rutin; **c.** Isoquercitrin; **d.** Caffeic acid; **e.** Chlorogenic acid; **f.** Ferulic acid.

➢ The TLC screening of phenolic compounds led to the identification of rutin, hyperoside, isoquercitrin, chlorogenic acid and caffeic acid, both in *C. monogyna* and *C. pentagyna* samples.

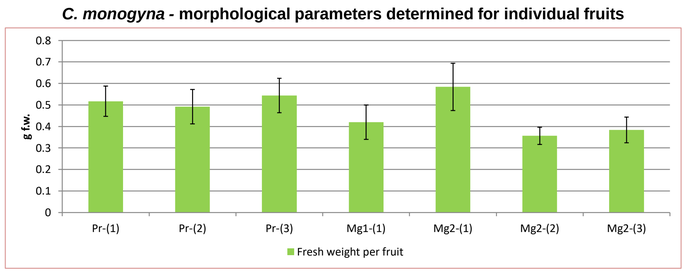
Phenolic compounds identified and quantified in *C. monogyna* (A) and *C. pentagyna* (B) samples



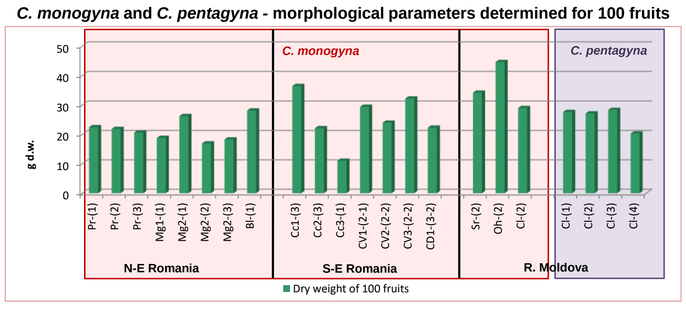
Hc1-HC4: Hydroxycinnamic acids; **CL:** Chlorogenic acid; **CF:** Caffeic acid; **VIT:** Vitexin; **RUT**: Rutin; **HYP:** Hyperoside; **IQR:** Isoquercitrin; **dQ:** Quercetin derivative; **K3G:** Kaempferol-3-glucoside

➢ Following the HPLC analysis, chlorogenic and caffeic acids, rutin, hyperoside and isoquercitrin were identified and quantified in all samples, while vitexin was present only in *C. monogyna* fruits.
➢ Hyperoside was the main phenolic compound in *C. monogyna* and isoquercitrin was the main phenolic compound in *C. pentagyna* samples.
➢ Overall, *C. monogyna* samples from R. Moldova are richer in phenolic compounds.

***C. monogyna* - morphological parameters determined for individual fruits**

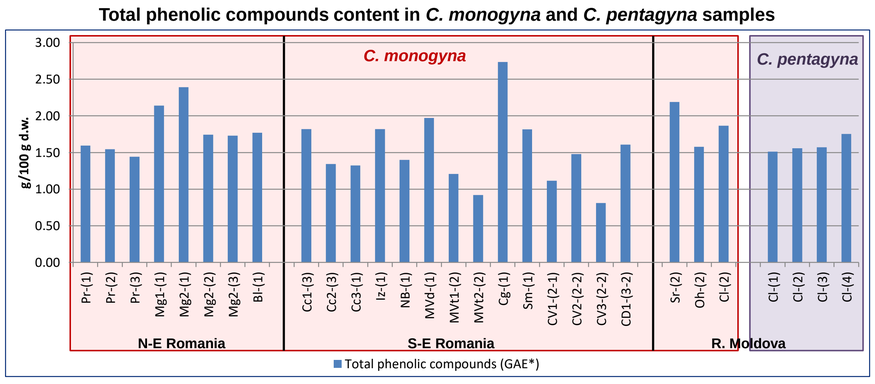


***C. monogyna* and *C. pentagyna* - morphological parameters determined for 100 fruits**



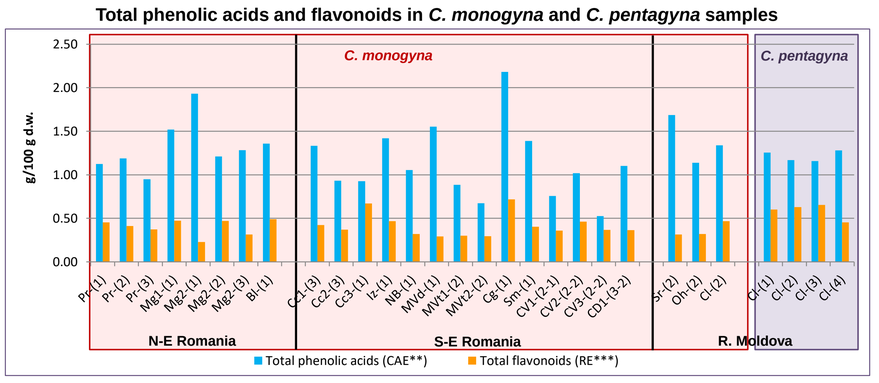
➢ For *C. monogyna* fruit samples, fresh weight per fruit varied between 0.36 ± 0.04 and 0.58 ± 0.11 g. Within the same sample, moderate to high variability was registered.
➢ Samples from Romania → moderate to high intrapopulation variability and high interpopulation variability for all morphological parameters. Samples from R. Moldova → moderate intrapopulation and interpopulation variability.
➢ Within the same area, the highest variability for dry biomass was observed for the S-E Romania (Dobrogea).
➢ When comparing the samples harvested from the 3 investigated areas, on average, the samples from R. Moldova had higher values for the dry biomass than those from Romania.

Total phenolic compounds content in *C. monogyna* and *C. pentagyna* samples



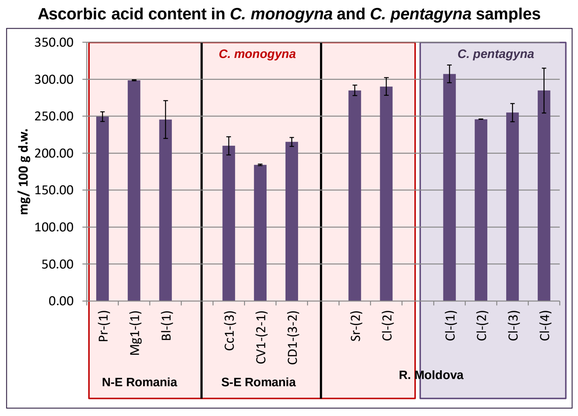
GAE*: Gallic acid equivalents; **CAE**:** Caffeic acid equiv.; **RE**: Rutin equiv.

Total phenolic acids and flavonoids in *C. monogyna* and *C. pentagyna* samples



➢ For both *Crataegus* species, the spectrophotometric evaluation of the total phenolic compounds (gallic ac. equiv.), phenolic acids (caffeic ac. equiv.) and flavonoids (rutin equiv.) content highlighted that, out of the total phenolic compounds, phenolic acids are predominant.

Ascorbic acid content in *C. monogyna* and *C. pentagyna* samples



➢ For *C. monogyna* total phenolic compounds content varied between 0.810 and 2.753 g/100 g GAE, phenolic acids content between 0.526 and 2.812 g/100 g CAE and flavonoids content between 0.228 – 0.719 g/100 g RE.
➢ For *C. pentagyna* total phenolic compounds content varied between 1.512 and 1.754 g/100 g GAE, phenolic acids content between 1.157 and 1.279 g/100 g CAE and flavonoids content between 0.453 – 0.655 g/100 g RE.
➢ Overall, moderate to high intrapopulation and interpopulation variability was observed for total phenolic compounds, phenolic acids and flavonoids.
➢ The highest variability was registered for the samples harvested from South-East region of Romania (Dobrogea) and the lowest one for the samples harvested from R. Moldova.

➢ Vitamin C content varied between 184.24 ± 0.96 and 298.59 ± 0.55 mg/100 g d.w. in *C. monogyna* samples, and between 245.87 ± 0.07 and 307.14 ± 11.93 mg/100 g d.w. in *C. pentagyna* samples.
➢ For *C. monogyna*, the samples from R. Moldova were richer in vitamin C and the lowest content was registered for the samples harvested from South-East Romania.

CONCLUSIONS

➢ On average, the *C. monogyna* samples from R. Moldova were richer in phenolic compounds and vitamin C than those from the Eastern half of Romania, the lowest content being registered for the samples from the South-East area of Romania.
➢ Regarding the samples from R. Moldova, *C. monogyna* fruits had higher phenolic compounds content compared to *C. pentagyna* fruits.
➢ For *C. monogyna*, the highest bioproductivity, both in terms of biomass and bioactive compounds content was observed for the different regions of R. Moldova.

References

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Acknowledgements

The work was sustained from the Nucleus Program (BIODIVERS 3 – 25N/19270401) financed by the Ministry of Research and Innovation (MCI), Romania, and supported by a grant of MCI, Program 1 – Development of the National R&D System, Subprogram 1.2 – Institutional Performance - Projects for Excellence Financing in RDI, Contract no. 22PFE/2018.

PSE E-CONGRESS 2020