Inhibition of digestive enzymes and antioxidant activity of extracts from fruits of *Cornus alba*, *Cornus sanguinea* subsp. *hungarica* and *Cornus florida* – a comparative study

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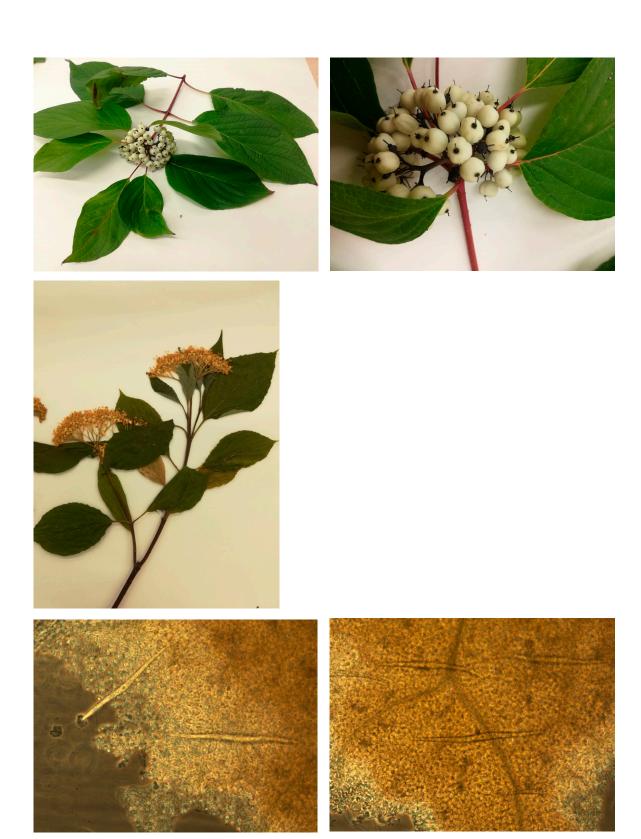


Figure S1. Morphological and anatomical characteristics of *Cornus alba* L. plant materials (leaves) in the optical microscope.



Figure S2. Morphological and anatomical characteristics of *Cornus sanguinea* L. subsp. *hungarica* plant materials (leaves) in the optical microscope.

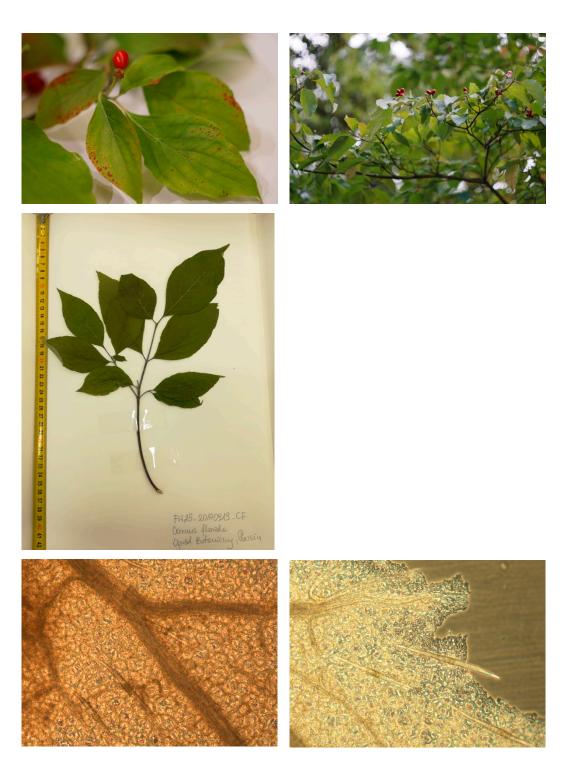


Figure S3. Morphological and anatomical characteristics of *Cornus florida* L. plant materials (leaves) in the optical microscope.

Corni fructus 1619g Acetone/MeOH/H₂O(3:1:1) 3L 4x24h (1h ultrasonic bath) evaporation to 1L Chloroform Diethyl ether 5x1L n-butanol Ethyl acetate 5x1L (1:1, v/v) 5 x 1L 5x1L evaporation evaporation evaporation evaporation Chloroform Diethyl ether Ethyl acetate Butanol fraction fraction fraction fraction 32g 32.9g 5.95g 50g Column chromatography: silica gel 6,5x25cm Elution - gradient: CHCI₃₍ (100%) - every 5%-> EtOAc (100%) EtOAc (100%) - every 10%->MeOH(100%) Fraction E (125-134) Fraction A Fraction B Fraction C Fraction D Fraction F Fraction G Fraction I (172-230) Fraction H (1-60) (141-157) (61-78) (79-98)(99-124) (135-140) (158-171)0,62g 2,53g `0,2g `17,51g 1,55g 2,21g 1,71g 3,08g 1,09g D5 D1 D2 D3 D4 H1 H2 Н3 H4 Н5 (48-70)(241-360) (1-24) (25-35)(36-39)(40-47)(1-60)(61-130) (131-140)(141-240)0,0411g 1,3786g 0,0295g 0,0440g 0,9360g 0,2713g 0,048g 5,5499 0,2011g D2.3 D2.4 D2.5 D2.1 D2.2 (14,50-14,80min.) (16,00-16,30min.) (17,35-17,75min.) (23,20-23,60min.) (28,00-28,50min.) 12,69mg 3,88mg 5,63mg D1.1 D1.2 D1.3 H1.1 75,86mg 8,75mg 12,66mg 6,66mg (17:30-18:00min.) (23,30-23,90min.) (24,40-24,80min.) (4,90-5,40min.)

Figure S4. The draft of isolation of compounds from acetone-methanol-aqueous (3:1:1, v/v/v) extract from fruit of *Cornus alba*.

NMR spectra of isolated compounds

1. Compound D2_1 (chlorogenic acid)

The compound D2_1 was identified as 5-O-coumaroylquinic acid. Its structure was confirmed based on the data available in the literature [1].

2. Compound D2_3 (5-O-coumaroylquinic acid)

¹H NMR (300 MHz, CD₃OD) δ 7.63 (d, J = 15.8 Hz, H-7'), 7.46 (d, J = 8.5 Hz, H-2',6'), 6.80 (d, J = 8.5 Hz, H-3',5'), 6.34 (d, J = 16.0 Hz, H-8'), 5.36 (s, H-5), 4.16 (s, H-3), 3.73 (dd, J = 3.6, 8.7 Hz, H-4), 2.16 (m, H-2), 2.10 (m, H-6).

The compound D2_3 was identified as 5-O-coumaroylquinic acid. Its structure was confirmed based on the data available in the literature [2].

3. Compound D2_4 (5-O-(E)-p-coumaroylquinic acid methyl ester)

¹H NMR (300 MHz, CD₃OD) δ 7.60 (d, J = 16.0 Hz, H-7'), 7.46 (d, J = 8.6 Hz, H-2', H-6'), 6.81 (d, J = 8.6 Hz, H-3', H-5'), 6.29 (d, J = 16.0 Hz, H-8'), 5.29 (ddd, J = 7.7, 7.7, 4.5 Hz, H-5), 4.14 (dt, J = 6.6, 3.4 Hz, H-3), 3.73 (dd, J = 7.6, 3.2 Hz, H-4), 2.19 (m, H₂-6), 2.02 (dd, J = 13.7, 6.7 Hz, H₂-6), 1.30 (s, OCH₃).

The compound D2_4 was identified as 5-*O*-(E)-*p*-coumaroylquinic acid methyl ester. Its structure was confirmed based on the data available in the literature [3].

4. Compound D2_5 (kaempferol 3-O-glucuronide 6"-methyl ester)

¹H NMR (300 MHz, CD₃OD) δ 8.01 (d, J = 8.9 Hz, H-2' e H-6'), 6.87 (d, J = 8.9 Hz, H-3' e H-5'), 6.40 (d, J = 1.9 Hz, H-8), 6.20 (d, J = 1.9 Hz, H-6), 5.23 (d, J = 7.3 Hz, H-1"), 3.65 (s, OCH₃), 3.60 – 3.34 (overlapping uronic acid moiety).

The compound D2_5 was identified as kaempferol 3-O-glucuronide-6"-methyl-ester. Its structure was confirmed based on the data available in the literature [4].

5. Compound H1_1 (hydroxytyrosol glucoside)

¹**H NMR** (300 MHz, CD₃OD) δ 6.62 (d, J = 3.1 Hz, H-2), 6.58 (s, H-5), 6.49 (d, J = 2.9 Hz, H-6), 4.31 (d, J = 7.7 Hz, H-1'), 4.05 (m, H-8a), 3.88 (d, J = 1.9 Hz, H-6'a), 3.79 – 3.65 (overlapping H-5', H-6'b, H-8b), 3.36 – 3.28 (overlapping H-2', H-3'), 3.19 (t, J = 8.3 Hz, H-4'), 2.87 (t, J = 7.3 Hz, H-7).

The compound H1_1 was identified as hydroxytyrosol glucoside. Its structure was confirmed based on the data available in the literature [5].

References

- 1. Nakamura, K.; Ogasawara, Y.; Endou, K.; Fujimori, S.; Koyama, M.; Akano, H. Phenolic compounds responsible for the superoxide dismutase-like activity in high-brix apple vinegar. *J. Agric. Food Chem.* **2010**, *58*, 10124-10132.
- 2. Lu Y., Sun Y., Foo L.Y. et al. 2000. Phenolic glycosides of forage legume *Onobrychis viciifolia*. *Phytochemistry* 55, 67-75.
- 3. Nakamura S., Fujimoto K., Matsumoto T., et al. 2013. Acylated sucroses and acylated quinic acids analogs from the flower buds of *Prunus mume* and their inhibitory effect on melanogenesis. *Phytochemistry*. 92, 128-36
- 4. Pacifico S., D'Abrosca B., Scognamiglio M., et al. 2013. Antioxidant polyphenolic constituents of *Vitis× Labruscana* cv. 'Isabella' leaves. *Open Nat Prod J.* 6, 5-11.
- 5. Romero C., Brenes M., García P., et al. 2002. Hydroxytyrosol 4-β-D-glucoside, an important phenolic compound in olive fruits and derived products. *J Agric Food Chem.* 50, 3835-9.