Phytochemical Investigation and Bioactivity of Extracts from the Artichoke "Carciofo di Procida", a Traditional Italian Agri-Food Product (PAT) of the Campania Region

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OVERVIEW

The globe artichoke (*Cynara cardunculus* L. subsp. *scolymus*) is a Mediterranean food plant increasingly valued for both its nutritional and medicinal properties: the edible part is represented by the immature inflorescence, but it is also recognized for therapeutic effects such as antioxidant, hepatoprotective, cholesterol-lowering, and antibacterial activities. Its health benefits are mainly attributed to polyphenols, especially mono- and dicaffeoyl quinic acids, which are typically abundant in extracts along with flavonoid glycosides of apigenin and luteolin. However, the composition and concentration of these metabolites vary significantly depending on factors such as genotype, cultivar, climate, soil, and growth stage, making the phytochemical profile highly dynamic.

"Carciofo di Procida" (*Cynara cardunculus L. subsp. scolymus*), a Traditional Italian Agri-food Product (PAT), cultivated in Procida (Gulf of Naples, IT)

OBJECTIVES

As part of our research on **bioactive metabolites from natural sources**, we analyzed the polyphenol and triterpenoid saponin content of the "Carciofo di Procida" cultivar, a Roman-type artichoke grown on Procida Island (Gulf of Naples, Italy) recently recognized as PAT and valued for its distinctive traits and long-standing cultivation using traditional methods.

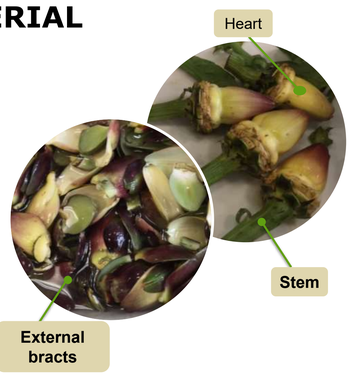
The chemical composition and biological properties of this cultivar have not been previously studied.

This work investigates its metabolite content in different parts including heart with internal bracts (H), external bracts (E), and stem (S), these latter two considered waste in food processing. Antioxidant properties and cytotoxic activities against four human tumoral cell lines were also evaluated for hydroalcoholic extracts.

METHODOLOGIES

BIOLOGICAL MATERIAL

The artichoke cultivar "Carciofo di Procida" (*Cynara cardunculus* L. subsp. *scolymus*) was cultivated in the spring of 2023 in a home-producing field in Procida, an island of the Gulf of Naples (Italy), in Solchiaro area (40°44'37" N. 14°00'38" E. 36 mt asl).



External bracts (E), hearts with inner bracts (H), and stems (S) were separated, stored frozen at −20 °C, and freeze-dried. For hearts and stems, six random samples were selected and extracted in technical replicate. External bracts were combined and extracted in triplicate

EXTRACTION STEP

✓ Hydroalcoholic extraction

Lyophilized material from six H and six S (in duplicate) and pooled E subsamples (in triplicate) were extracted with EtOH:H_2O (1:1) (6 mL × 2) under shaking for 1 h at 38 °C at 100 rpm.

✓ Extraction with acidified solvents

Lyophilized samples of the different tissues were extracted with 1 mL of MeOH/H_2O/TFA (70:30:0.5, v/v/v) in an orbital shaker at 100 rpm for 1 h at room temperature.

CHEMICAL AND BIOLOGICAL ANALYSES

- ✓ Total Polyphenol Content (TPC)
- ✓ Quali-quantitative profiling of Polyphenols and Saponins by UHPLC-ESIMS$^-$
- ✓ Quali-quantitative profiling of Anthocyanins by UHPLC-ESIMS$^+$
- ✓ Antioxidant Activity (DPPH and FRAP assays)
- ✓ Cytotoxicity on normal and tumoral cell lines (MTT assay)

- ✓ Total Anthocyanin Content (TAC)

RESULTS

Chemical analysis

❑ *LC-MS/MS profile of polyphenols, saponins, and anthocyanins* ❑ *Total polyphenol content* ❑ *Total anthocyanin content*

Compound	Exact Mass	Measured *m/z*	MS/MS	Rt	H mg/100 g DW	H SD	E mg/100 g DW	E SD	S mg/100 g DW	S SD
Chlorogenic acid	353.0873	353.0888	191.0556	1.85	35.62 ^{b}	3.67	43.7 ^{b}	1.9	153.11 ^{a}	59.71
p-coumaroyl quinic acid	337.0934	337.0938	191.0556	2.24	31.59 ^{a}	3.57	8.94 ^{c}	0.43	15.03 ^{b}	3.30
3,5-dicaffeoyl quinic acid	515.1195	515.1204	353.0882/191.0556	3.23	5.84 ^{b}	1.26	9.04 ^{b}	0.66	31.91 ^{a}	15.69
Luteolin-7-*O*-rutinoside	593.1506	593.1522	285.0408	2.78	0.30 ^{b}	0.09	1.16 ^{a}	0.09	1.74 ^{a}	0.84
Luteolin-7-*O*-glucuronide	461.0720	461.0735	285.0409	2.96	31.39 ^{a}	3.46	19.03 ^{b}	0.52	4.45 ^{c}	0.89
Luteolin-7-*O*-glucoside	447.0933	447.0938	285.0409	2.98	2.19	0.46	3.76	0.30	2.70	1.30
Apigenin-7-*O*-rutinoside	577.1563	577.1570	269.0460	3.26	8.04 ^{a}	1.50	3.35 ^{b}	0.17	0.25 ^{c}	0.02
Apigenin-7-*O*-glucoside	431.0978	431.0987	269.0460	3.49	20.80 ^{a}	2.31	3.38 ^{b}	0.17	1.42 ^{b}	0.19
Apigenin-7-*O*-glucuronide	445.0776	445.0782	269.0460	3.52	103.95 ^{a}	5.00	71.52 ^{b}	2.74	45.82 ^{c}	3.67
Apigenin	269.0455	269.0458	151.0026/117.0332	5.72	1.72 ^{b}	0.38	1.10 ^{b}	0.40	13.14 ^{a}	3.30
Cynarasaponin J	941.4752	941.4767	779.4255/629.3701/471.3501	5.71	11.64 ^{b}	3.56	<LOQ		193.68 ^{a}	45.59
Cynarasaponin F/I	779.4223	779.4237	717.4263/629.3701/471.3501	5.95	17.48 ^{b}	4.46	<LOQ		108.21 ^{a}	27.59
Cynarasaponin E	809.4329	809.4336	647.3750/471.3501	6.11	1.11 ^{b}	0.42	<LOQ		69.84 ^{a}	24.27
Cynarasaponin A/H	925.4832	925.4818	763.4285/613.3754/455.3527	6.57, 6.84	29.02 ^{b}	9.24	2.02 ^{b}	0.09	891.90 ^{a}	149.11

Quantitative analysis of polyphenols and saponins measured as $[M-H]^-$ ions in H, E and S extracts. Results are reported as mg/100 g dry weight (DW), mean values ± standard deviation (SD). LOQ = limit of quantitation, referred to the lowest point of the calibration curve at 30 ng/mL. Luteolin was detectable but always below the LOQ. Different letters (a, b, c) indicate statistically significant differences at $p < 0.05$ (one-way ANOVA) among H, E, and S.

Compound	Exact Mass	Measured *m/z*	MS/MS	Rt	H mg/100 g DW	H SD	E mg/100 g DW	E SD	S mg/100 g DW	S SD
Cyanidin-malonylglycoside	535.1082	535.1088	287.0549	3.52	0.39 ^{b}	0.22	2.25 ^{a}	0.03	1.98 ^{a}	0.97
Cyanidin-malonylglycoside 2	535.1082	535.1088	287.0549	3.63	0.10 ^{b}	0.08	2.39 ^{a}	0.43	nd	
Peonidin-glycoside	463.1235	463.1239	301.0708	3.69	nd		0.68	0.33	nd	
Peonidin-malonilglycoside	549.1239	549.1250	301.0708	4.29	nd		0.56	0.43	nd	
Peonidin-glycoside 2	463.1235	463.1239	301.0708	4.99	0.21 ^{b}	0.06	0.85 ^{a}	0.09	nd	

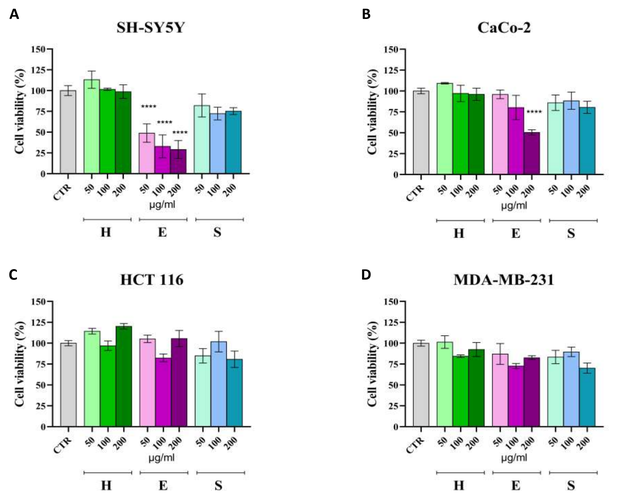
Quantitative analysis of main anthocyanins measured as $[M]^+$ ions in H, E and S extracts of artichoke cultivar "Carciofo di Procida". Results are reported as mg eq cyanidin-3-O-glucoside/100 g dry weight (DW), mean values ± SD; nd—not detected. Different letters (a, b) indicate statistically significant differences at $p < 0.05$ (one-way ANOVA) among H, E, and S.

Biological analysis

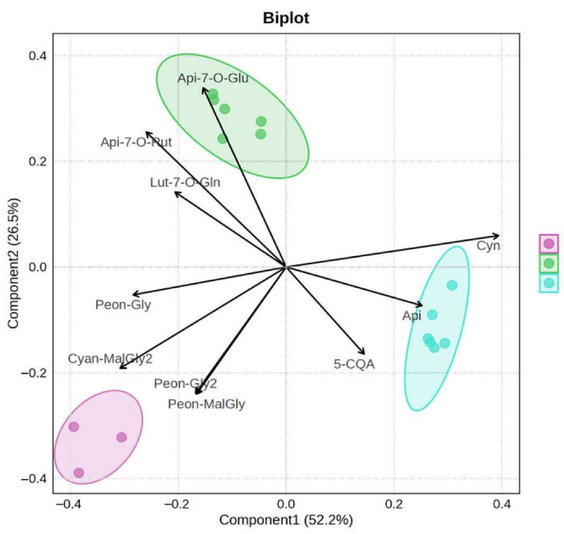
❑ *Free Radical-Scavenging assay* ❑ *Ferric Reducing Power Activity assay* ❑ *MTT Cytotoxicity assay*

	TPC mg/g DW	TAC mg/ 100 g DW	DPPH g eq TROLOX/ 100 g DW	DPPH IC_{50} µg/ml	FRAP µmol Fe^{2+} eq/ 100 g DW
H	1.20 ± 0.06^b	1.50±0.04^b	3.01 ± 0.01^b	73.89 ±2.61^a	2118.44 ±0.90^a
E	3.40 ± 0.20^a	12.70±0.02^a	5.88 ± 0.04^a	37.34 ±0.54^c	1802.28 ±1.07^c
S	0.75 ± 0.04^c	-	2.66 ± 0.01^c	69.23 ±1.24^b	1890.38 ±1.56^b

Total polyphenol content (TPC), monomeric anthocyanin content (TAC), and antioxidant activity of artichoke extracts of heart with inner bracts (H), external bracts (E) and stems (S). Values are expressed as mean ± standard deviation. Different letters (a, b, c) indicate statistically significant differences at $p < 0.05$ (one-way ANOVA).



MTT assay for cytotoxicity determination: bar plot representing cell viability (%) of cells treated with different concentrations of artichoke extract (50, 100, and 200 µg/mL) after 24 h. (A) SH-SY5Y cell line; (B) CaCo-2 cell line; (C) HCT 116 cell line; (D) MDA-MB-231 cell line. Statistical analysis was performed using ANOVA for multiple comparisons (**** $p < 0.001$).



PLS-DA biplot showing the separation of the three artichoke parts (H, E, and S) based on their metabolic profiles. The clear distinction among the groups indicates significant metabolic differences, with the separation of group E primarily driven by anthocyanins.

KEY FINDINGS

- The **most abundant flavonoid** in artichoke **heads** is **Apigenin-7-O-Glucuronide.** Together with the other two glycosides, i.e., rutinoside and glucoside, apigenin derivatives account for 132.80 and 78.25 mg/100 g DW of H and E, respectively. **Luteolin derivatives**, including its glucosides, rutinoside, and glucuronide, **are less abundant** (33.88 and 23.95 mg/100 g DW in H and E, respectively). **Chlorogenic acid** is the **most abundant among the quinic acid derivatives in head** sections extracts, but it has been recovered in a **larger amount in stem** (153.11 mg/100gDW).
- The **dominant metabolites in stem** are the **triterpenoid glycosides cynarasaponins**, especially cynarasaponin A which represents about 1% of the stem dry material.
- **Anthocyanins** were **mainly recovered in external bracts.**
- **External bracts** extracts exhibited **the highest TPC** (3.4 ± 0.2 mg/g DW) and **radical scavenging activity** (5.88 ± 0.04 g/100 g DW);
- A **marked reduction in cell viability** was observed **on neuroblastoma SH-SY5Y** cancer **cell lines** treated with **extracts from external bracts** (50 µg/mL, $p < 0.001$);
- **Anthocyanins,** namely peonidin and cyanidin glycosides, emerged as **key discriminant compounds** by **PLS-DA** analysis **correlating metabolic profiles and cytotoxicity;**
- **Non-edible parts of this cultivar** are **a valuable source of bioactive material.**

Tommonaro, G.; De Simone, G.; Iodice, C.; Allarà, M.; Cutignano, A. A Phytochemical and Biological Characterization of Cynara cardunculus L. subsp. scolymus Cultivar "Carciofo di Procida", a Traditional Italian Agri-Food Product (PAT) of the Campania Region. Molecules 2025, 30, 3285