Date:
12.01.2026

Application Note — Propolis SFE-EtOH and Subcritical Water Extracts

Sample information

Sample names: Orange Propolis (OLD), Black Propolis (ErkanBey)

Sample type: Two raw propolis samples extracted by two complementary methods: co-solvent assisted supercritical CO_2 with 15% v/v ethanol (SFE-EtOH) and subcritical water extraction (SWE). Both samples are dried, granulated raw propolis.

Scientific / botanical origin: Resinous bee-collected exudate; botanical origin varies by region (typically *Populus*, *Pinus* and other resin-producing flora)

Number of samples: 2 raw materials × 2 extraction methods = 4 extract samples

Number of analyses: n = 1 per extract for yield, TPC, TFC and DPPH; calibration $R^2 \geq 0.99$ for reported DPPH IC_{50} values

Sample preparation and analytical procedures

Analysis date: January 2026

Analyst: Dr. Deniz Can Köseoğlu

Sample preparation: Raw materials weighed, dried to constant weight where applicable (moisture content recorded), and milled or ground prior to loading the extraction vessel. Extract fractions collected, weighed, and dissolved in methanol for spectrophotometric assays.

SFE-EtOH method: Co-solvent assisted $scCO_2$ extraction at **350 bar / 50 °C**, 15% v/v food-grade ethanol as co-solvent, dynamic mode, total extraction time **180 min**. Extract collected through a single decompression separator; co-solvent volume collected recorded for solvent-to-material (S/M) ratio.

SWE method: Subcritical water extraction at **140 °C / 40 bar**, dynamic mode, total extraction time **30 min**. Water-soluble extract recovered after depressurisation; aqueous extract freeze-dried for spectrophotometric and yield analysis.

TPC method: Folin–Ciocalteu protocol; absorbance at 765 nm against a gallic acid calibration curve. Results in mg gallic acid equivalents (GAE) per g dry extract.

TFC method: Aluminium chloride ($AlCl_3$) colorimetric assay; absorbance at 415 nm against a quercetin calibration curve. Results in mg quercetin equivalents (QE) per g dry extract.

DPPH method: DPPH radical scavenging activity (RSA, %) measured at seven extract concentrations (10–120 ppm). IC_{50} obtained by linear interpolation of RSA versus concentration at the 50 % scavenging level; only IC_{50} values with calibration $R^2 > 0.99$ are reported as reliable. Lower IC_{50} indicates stronger antioxidant activity.

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Extraction conditions

Two complementary extraction techniques were applied to each raw material in parallel runs. The **SFE-EtOH** method uses supercritical CO_2 with ethanol as a polar co-solvent, optimised for lipophilic and moderately polar phenolics. The **SWE** method uses water above its atmospheric boiling point but below the critical point, where the dielectric constant drops sharply and the medium behaves like an organic solvent — well-suited for highly polar phenolics, tannins and water-soluble flavonoids. The conditions summarised below were held constant for every run in this campaign.

Parameter	SFE-EtOH	SWE
Pressure	350 bar	40 bar
Temperature	50 °C	140 °C
Co-solvent / medium	15 % v/v ethanol (in $scCO_2$)	Pure water (subcritical)
Extraction time	180 min	30 min
Mode	Dynamic	Dynamic

Extraction yields and material balance

Sample	Method	Loading (g)	Moisture (%)	Extract (g)	Yield (% dry basis)	Co-solvent collected (mL)	S/M ratio
Orange Propolis	SFE-EtOH	97.97	0.00	75.52	77.08	2085	21.28
Orange Propolis	SWE	18.30	0.00	10.38	56.74	1385	75.70
Black Propolis	SFE-EtOH	201.09	2.61	22.00	11.23	1640	8.37
Black Propolis	SWE	179.09	2.61	14.62	8.38	910	5.22

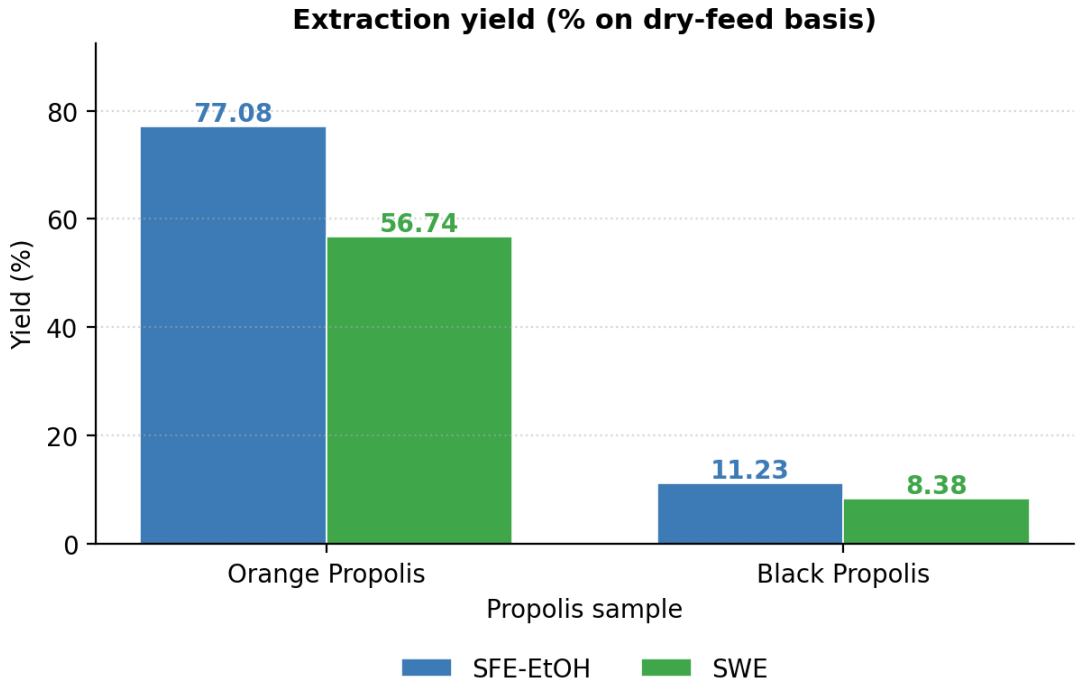

Figure 1. Extraction yield (% on dry-feed basis) for each propolis sample by extraction method.

Results — Total Phenolic and Flavonoid Content

Sample	Method	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	DPPH IC_{50} (ppm)	R^2 (DPPH)
Orange Propolis	SFE-EtOH	67.32	5.61	n/a	n/a
Orange Propolis	SWE	301.98	18.27	22.99	0.997
Black Propolis	SFE-EtOH	131.30	279.21	107.89	0.993
Black Propolis	SWE	191.12	222.38	53.90	0.990

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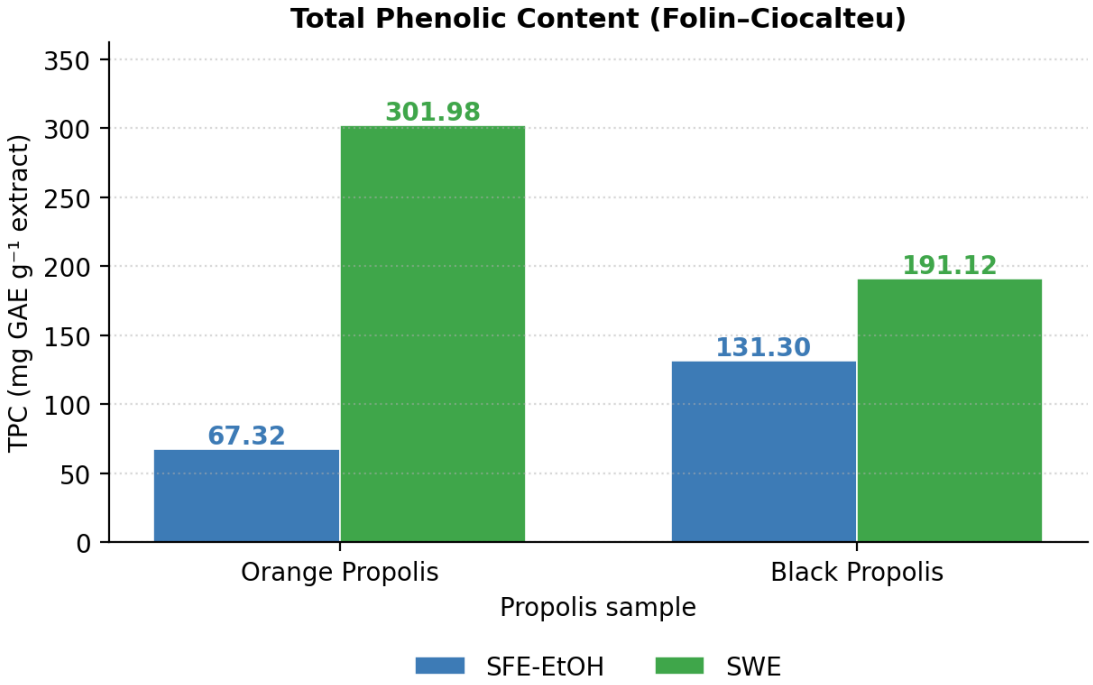

Figure 2. Total phenolic content (TPC) of each propolis extract.

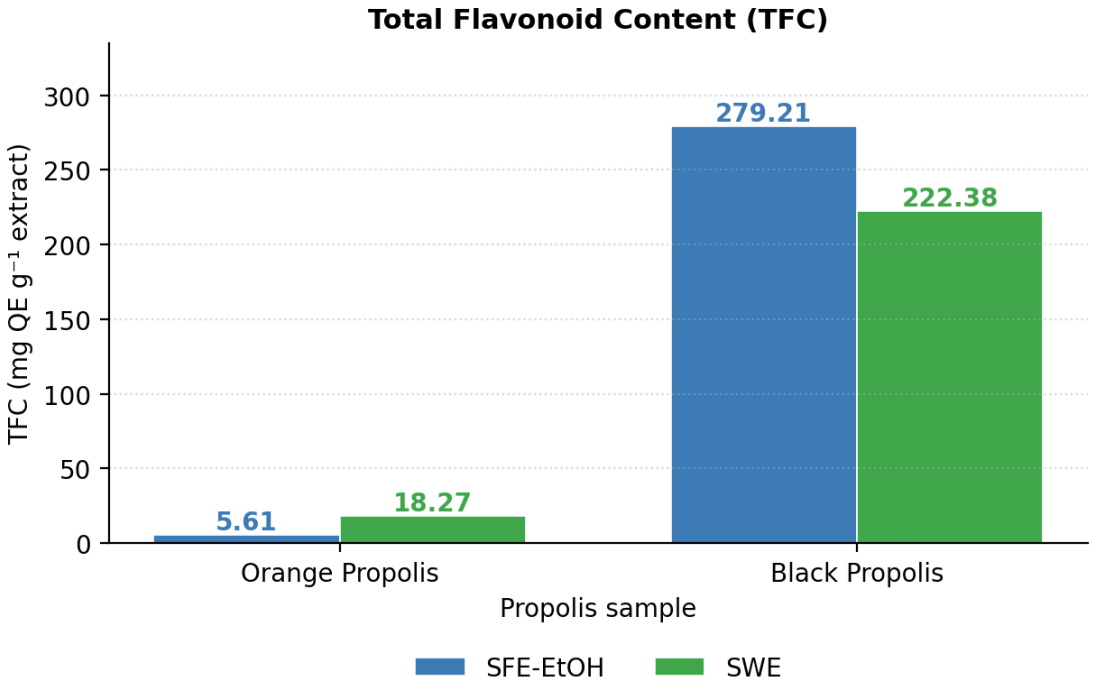

Figure 3. Total flavonoid content (TFC) of each propolis extract.

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Results — DPPH antioxidant activity

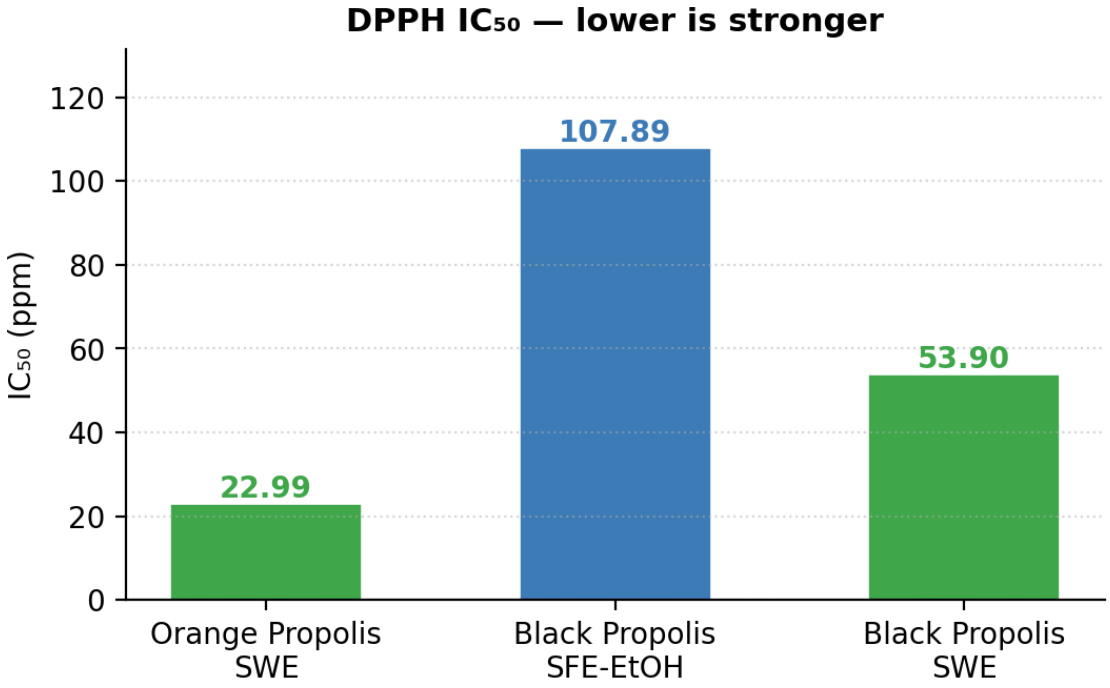

Figure 4. DPPH IC_{50} of reliably-quantified propolis extracts (R^2 > 0.99).

Discussion

The two propolis samples show **fundamentally different extraction responses** to SFE-EtOH versus SWE. Orange Propolis (OLD), with no measured moisture, delivered an exceptionally high SFE-EtOH yield of 77.08 % — characteristic of a resin matrix where the bulk of the extractable mass consists of waxes, resin acids and lipophilic constituents that $scCO_2$ + EtOH can readily solubilise. Black Propolis (ErkanBey), in contrast, gave a much lower SFE-EtOH yield (11.23 %), suggesting a higher proportion of polar, water-soluble constituents or a tighter resin matrix that resists CO_2 penetration.

On a phenolic-content basis the picture inverts. SWE consistently produces extracts with markedly higher TPC: 301.98 vs 67.32 mg GAE g^{-1} for Orange Propolis, and 191.12 vs 131.30 mg GAE g^{-1} for Black Propolis. The SWE medium at 140 °C selectively dissolves polar phenolics and flavonoids that the lipophilic SFE-EtOH stream cannot reach efficiently — the *concentration* of phenolics in the dry SWE extract is therefore 1.5–4.5× higher even though the absolute extract mass is smaller.

Black Propolis stands out as the **flavonoid-rich material**: TFC reaches 279.21 mg QE g^{-1} in the SFE-EtOH extract and 222.38 mg QE g^{-1} in the SWE extract — two orders of magnitude above the Orange Propolis values. This is consistent with the higher abundance of flavonoid aglycones in poplar-type propolis from cooler-climate flora.

DPPH IC_{50} results (reliable values only, R^2 > 0.99) show that the SWE extracts of both propolis types are the stronger antioxidants. Orange Propolis SWE gives IC_{50} = 22.99 ppm — the strongest activity in the dataset — followed by Black Propolis SWE at 53.90 ppm and Black Propolis SFE-EtOH at 107.89 ppm. The Orange Propolis SFE-EtOH IC_{50} could not be reliably determined (R^2 below the acceptance threshold).

Practical recommendation: for total-phenolic and antioxidant-driven applications (food preservation, supplement actives, antioxidant standardisation) SWE is the preferred route for both propolis types. For lipophilic, wax/resin-rich extracts intended for topical or cosmetic use, SFE-EtOH on Orange Propolis is the high-yield option. The two methods are complementary rather than competing — they recover distinct phytochemical fractions of the same raw material.

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