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Propolis Preparation and chemical analysis

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We use this protocol and it's working

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1 Propolis was collected from an apiary located at the Faculty of Agriculture, Cairo University (Giza province, Egypt).

2 The collected propolis was extracted for a week with 70 % ethanol at room temperature.

3 Extracted propolis was filtrated and then ethanol was removed by vacuum evaporator at 50°C.

4 The extracted propolis was kept as a powder form in a dark bottle at 4°C until use in the experiment.

5 Extracted propolis was mixed with a solution of 0.1 mM of 1,1- diphenyl-2-picryl-hydrazil (DPPH) in methanol, at different concentrations (25 to 75 µg/mL).

6 The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min.

7 The free radical scavenging activity was measured as the absorbance of reactions using an automatic spectrophotometer at 517 nm.

8 A high-performance liquid chromatography (HPLC) was achieved at 25°C on an Agilent 1260 Infinity HLPC Series (Agilent, Santa Clara, CA) equipped with Quaternary pump, Zorbax Eclipse plus C18 column 150 mm × 4.6 mm internal diameters, and 5 µm particle (Agilent).

9 A ternary linear elution profile was performed by HLPC gradient water in 0.2 % H3PO4 (v/v), methanol and acetonitrile.

10 The injected volume was 20 µL and the variable wavelength detector (VWD) was set at 284 nm for detecting the phenolic acids and flavonoid contents in the extracted propolis.