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**Modern and traditional extraction techniques affect chemical composition and
bioactivity of *Tanacetum parthenium* (L.) Sch.Bip**

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Accelerated solvent extraction (ASE)

An ASE 350 system Dionex Corporation (Sunnyvale, CA, USA) was used for the pressurised liquid extraction. One gram of herbal samples was mixed with 250 mg diatomic earth in 22 mL cells equipped with a stainless steel frit and a cellulose filter at the bottom to avoid the collection of suspended particles in the collection vial. The extracts were prepared using ethanol as a solvent and performed at 1500 psi and temperature of 120 °C, and then heated for 6 min, applying one extraction cycle of 5 min. The cells were rinsed with fresh extraction solvent (30% of the extraction cell volume) and purged with N₂ gas for 30 s and extracts were collected into 50 mL tubes.

Microwave-assisted extraction (MAE)

Five grams of plant sample was extracted with 100 mL of 96% (w/w) ethanol (1:20 ratio). The extractions were performed in an open system at 600 W during 30 min.

Maceration

To produce macerated extracts, the plant samples (5 g) were macerated with 100 mL of ethanol at room temperature (at dark) for 24 h.

Soxhlet extraction (SE)

Five grams of powdered plant were separately extracted with ethanol (100 mL, 96%, w/w) in a Soxhlet apparatus for 6 h.

Ultrasonication-assisted extraction (UAE)

The powdered plant (2 g) was extracted with 50 mL of ethanol (96%, w/w) for 60 min in a sonication bath at 30 °C. The temperature changes were monitored using. Whenever an increase in temperature, fresh water was circulated to maintain the temperature at 30 °C. To avoid the evaporation of ethanol, the beaker was covered with aluminum foil.

The obtained extracts were concentrated under vacuum at 40 °C by using a rotary vacuum evaporator. All samples were stored at 4°C in the dark until use.