

Ultrasound-assisted extraction of bioactive compounds from a medicinal plant: Impact on phenolic content and antioxidant capacity

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INTRODUCTION & AIM

- Plant therapeutic properties are related with the formation of secondary metabolites such as flavonoids, tannins, terpenoids, and alkaloids [1]. These natural products possess high scavenging capacity due to the presence of hydroxyl groups, which reduce oxidative stress [2].
 - Numerous extraction methods can be used to recover valuable bioactive compounds from plant material. However, the selection of the appropriate extraction technique is mainly based on the investment cost and extraction yield [3].
 - The conventional extraction techniques, such as Soxhlet, heating reflux, and maceration, are energy consuming, require high solvent demand, and a long extraction time resulting to thermal degradation of polyphenols which affects the extraction yield [4], [5].
 - More sustainable and greener techniques can be applied to overcome these limitations, including ultrasound-assisted extraction (UAE), supercritical extraction, and microwave-assisted extraction [4], among others, the use of UAE is considered as an efficient, economically, and environmentally viable method [6].
 - Verbascum sinuatum* (V. sinuatum) is a plant belonging to the Scrophulariaceae family that has been used in traditional medicine for the treatment of many diseases. Studies have shown that V. sinuatum has a higher phenolic and flavonoid content than other species in the same genus [7].
 - UAE presents a rapid and efficient method for producing high-quality antioxidant agents. As reported for various *Verbascum* species, UAE reduces extraction time from several hours or days to just minutes compared to maceration [8], [9].
- The aim of this study was to extract bioactive compounds from V. Sinuatum leaves via UAE using different solvents to evaluate their impact on the concentration of Total Phenolic Compounds (TPC) and to compare the antioxidant capacity of different extracts.

MATERIALS & METHODS

- V. sinuatum leaves were collected from the northern region of Algeria, washed several times with distilled water, and air-dried in the dark at room temperature for 4 weeks.
- The extraction was conducted using a 5 L ultrasonic cleaner (GMF Medical System Gmb, Graf-Adolf-Platz 15, 40213 Düsseldorf, Germany) with a working frequency of 40 KHz.
- For extraction, the ground plant material was put into a flask containing selected solvent, at a sample-to-solvent ratio of 1/30 (w/v). The extraction temperature, ultrasonic power, and extraction time were controlled from the panel of the instrument and set as 50 °C, 120 W, and 40 min, respectively [10].
- After sonication, the suspension was vacuum filtrated through a Whatman No. 2 filter paper. The supernatant was collected and concentrated under vacuum at 40°C using a rotary evaporator (Heidolph, Schwabach, Germany) (Fig. 1). The crude extract was then recovered with the appropriate solvent and stored at 4 °C until further analysis.

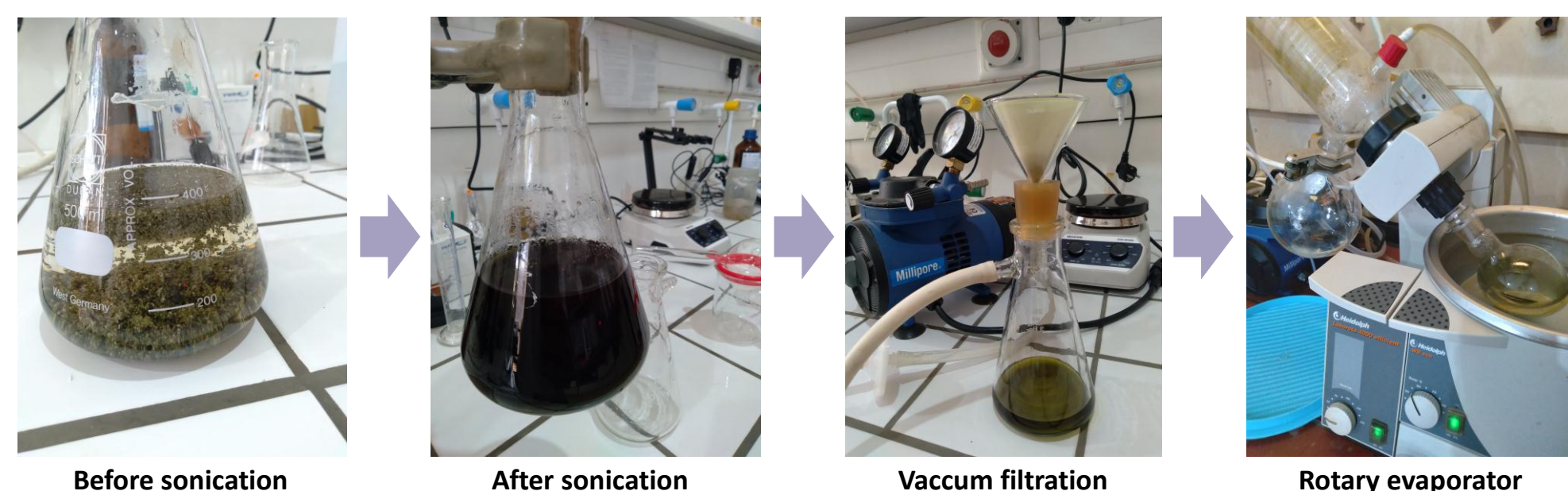


Fig. 1 Schematic representation of the extraction procedure

- The Folin–Ciocalteu method and the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay were used to estimate the concentration of total phenolic compounds and antioxidant activity, respectively [11], [12].

RESULTS & DISCUSSION

- The concentration of Total Phenolic Compounds increased in the following order of solvents (Fig. 2): water < 100% methanol < 100% ethanol < 50% methanol < 50% ethanol
- Slightly higher concentrations were observed when using pure alcohol as a solvent compared to pure water, which can be attributed to the degradation of lipid cell membranes and the subsequent release of compounds from plant cells [13]. Close results were obtained with the ethanolic and methanolic extracts, which could be explained by their relatively close polarity indices of 5.2 and 6.6, respectively [14].
- The 50% ethanol (v/v) was the most efficient solvent in extracting polyphenols. Highly polar solvents are capable of extracting a broader range of compounds with varying polarities. Consequently, an increase in water content enhances polarity compared to absolute ethanol [15].

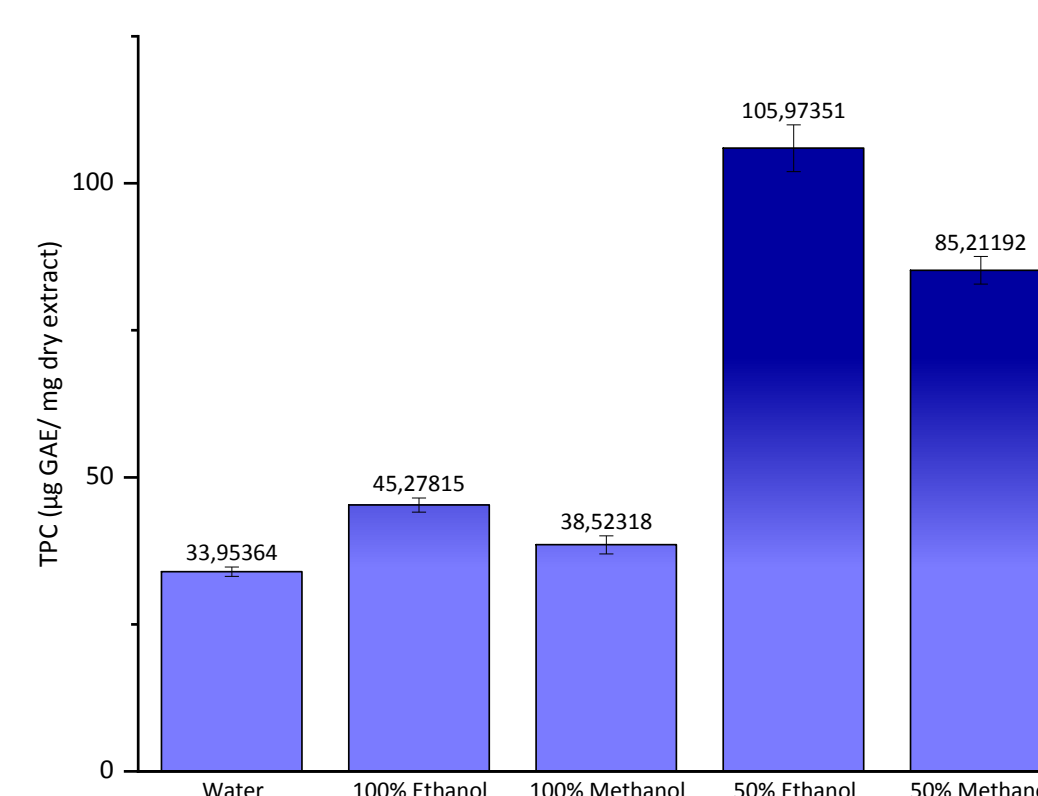


Fig. 2 Total phenolic compounds of different extracts

- The inhibition percentage of DPPH of all the extracts (Fig. 3) showed concentration-dependent activity profiles. The IC 50 values of all the samples decrease in this order: 100% ethanol > 50% ethanol > 50% methanol > Ascorbic acid. Lower IC 50 values are associated to higher antioxidant activity [16].

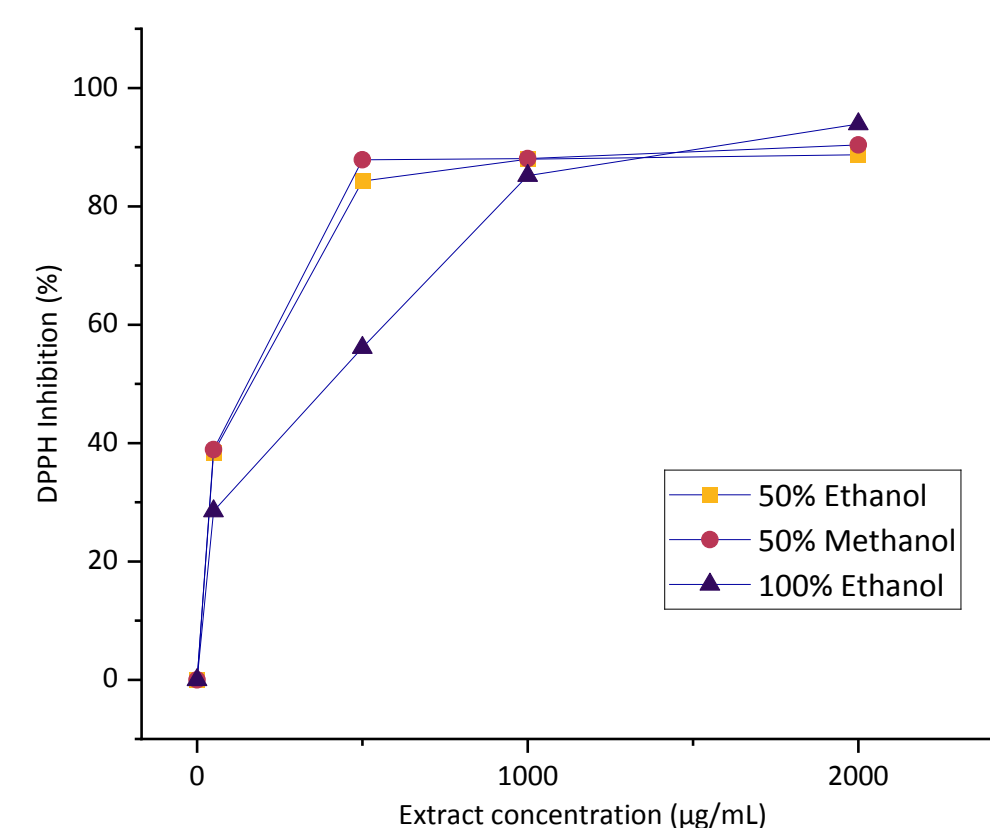


Fig. 3 DPPH radical scavenging activity and IC 50 values of plant extracts

	IC 50 (µg/ml)
Ascorbic acid	72.75 ± 0.30
50 % Ethanol	163.65 ± 0.08
50 % Methanol	152.07 ± 0.43
100 % Ethanol	400.26 ± 0.21

Data presented are mean ± SD

- The 50% methanol extract and the 50% ethanol extract exhibited almost identical antioxidant activity, despite the methanolic extract having a lower total phenolic content compared to the ethanolic extract. This could likely be attributed to the presence of non-phenolic compounds [15].

CONCLUSION

- The results showed that the total phenolic content was influenced by the polarity of the solvents. The highest TPC value (105.97 ± 3.97 µg GAE/mg dry extract) was obtained with 50% ethanol, which was selected as the most efficient solvent. Although 50% methanol exhibited slightly higher antioxidant capacity, 50% ethanol was preferred due to its lower toxicity compared to other solvents, such as methanol.

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