

Optimization of Green Ultrasound-Assisted Extraction of Phenolic
Compounds from a Species of the Scrophulariaceae FamilyMonsef Merdjemak*¹, Hadjer Mabrouki*¹, Rosa Haouche¹, Djamal Eddine Akretche¹¹Laboratory of hydrometallurgy and Inorganique Molecular Chemistry, Faculty of Chemistry. University of Sciences and Technology Houari Boumediene USTHB, BP 32, El-Alia 16111, Bab-Ezzouar, Algiers, Algeria.

INTRODUCTION & AIM

- Ancient populations have utilized plants for their nutritional and therapeutic benefits since ancient times [1].
- Algeria is the largest country in Africa and is renowned for its diverse climate. Its vast territory and strategic position in the southern Mediterranean basin have contributed to the growth of a highly diversified flora, making it one of the countries with the richest biodiversity in North Africa [2], [3].
- More than 3139 species from 150 families, including 653 endemic species, were found in Algeria [4].
- Secondary metabolites such as flavonoids, terpenoids..., possess high antioxidant capacity due to the presence of hydroxyl groups [5], [6].
- *Verbascum sinuatum* (Scrophulariaceae family) is a plant that has been used in traditional medicine for the treatment of many diseases [7].
- Ultrasound-assisted extraction (UAE) is considered as an efficient, economically, and environmentally viable method [8].
- The passage of ultrasonic waves through the solvent creates acoustic cavitation, causing cell disruption, allowing greater solvent penetration, thus increasing mass transfer [9].
- The aim of this study was to extract bioactive compounds from *Verbascum sinuatum* (V. sinuatum) leaves via UAE under different conditions to evaluate the impact of each factor on the concentration of Total Phenolic Compounds (TPC).

MATERIALS & METHODS

- Fresh *V. sinuatum* leaves were collected from the northern region of Algeria, washed with distilled water, and air-dried in the dark at room temperature for 4 weeks. After that, the plant samples were ground using an electric grinder, stored in hermetically sealed glass bottles, and kept away from moisture. The botanical identification was confirmed by a botanist at the Faculty of Biology, University of Science and Technology Houari Boumediene, Algeria.
- The extraction of bioactive compounds 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, 120 W (Figure. 1).

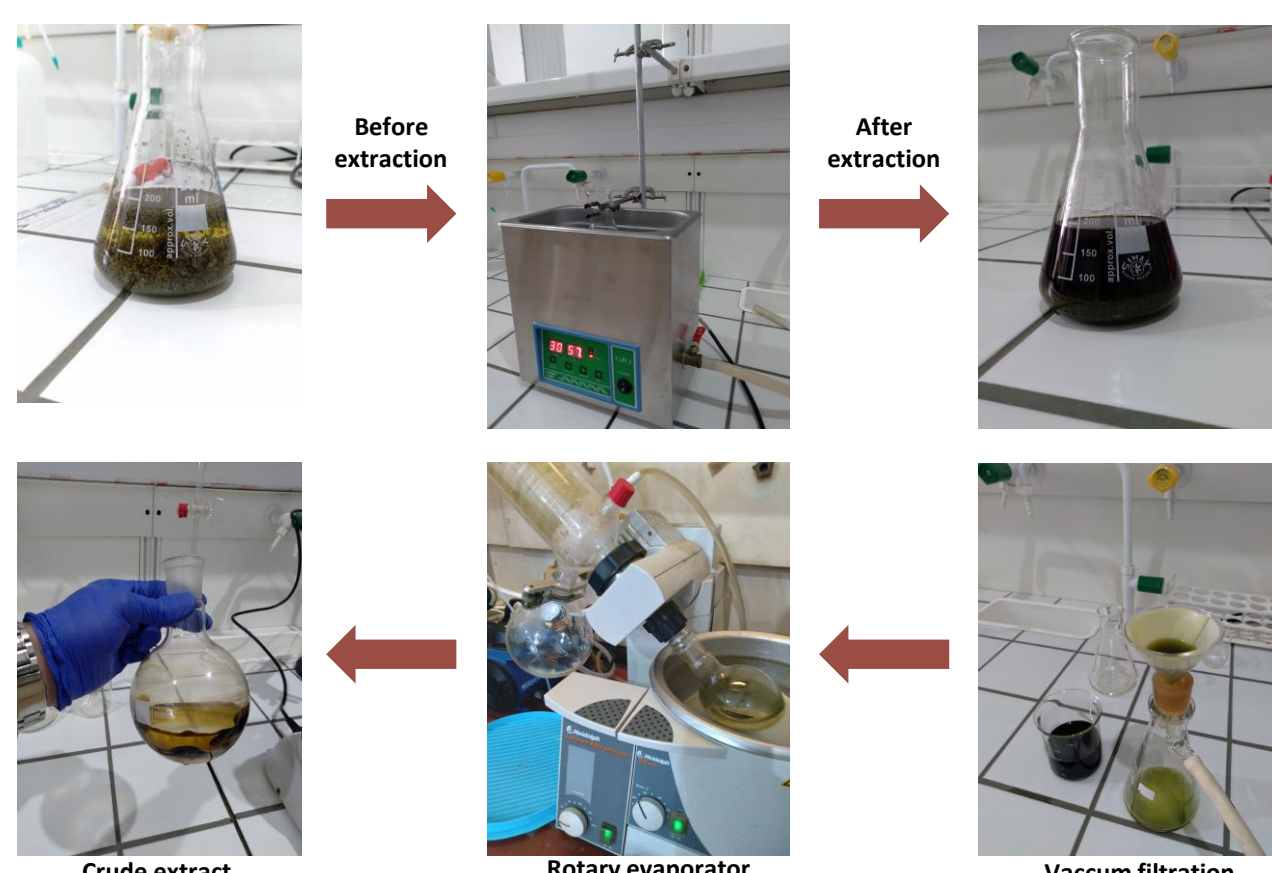


Figure. 1 Schematic representation of the extraction procedure

- To assess the individual impact of each parameter on TPC, a single-factor experimental approach was employed, where each factor was varied individually while the others were kept constant (Table. 1).

Table. 1 Single-factor experiments

	Temperature (°C)	Time (min)	Ratio (ml/g)	Solvent concentration (%)	Ultrasonic power (%)
A. Temperature	[30 – 80]	40	30	90	100
B. Time	50	[20 – 120]	30	90	100
C. Ratio	50	40	[30 – 60]	90	100
D. Solvent concentration	50	40	30	[40 – 90]	100
E. Ultrasonic power	50	40	30	90	[50 – 100]

- The Folin–Ciocalteu method was used to evaluate Total Phenolic Compounds (TPC) of samples according to Lefahal et al. [1] with slight modifications.

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RESULTS & DISCUSSION

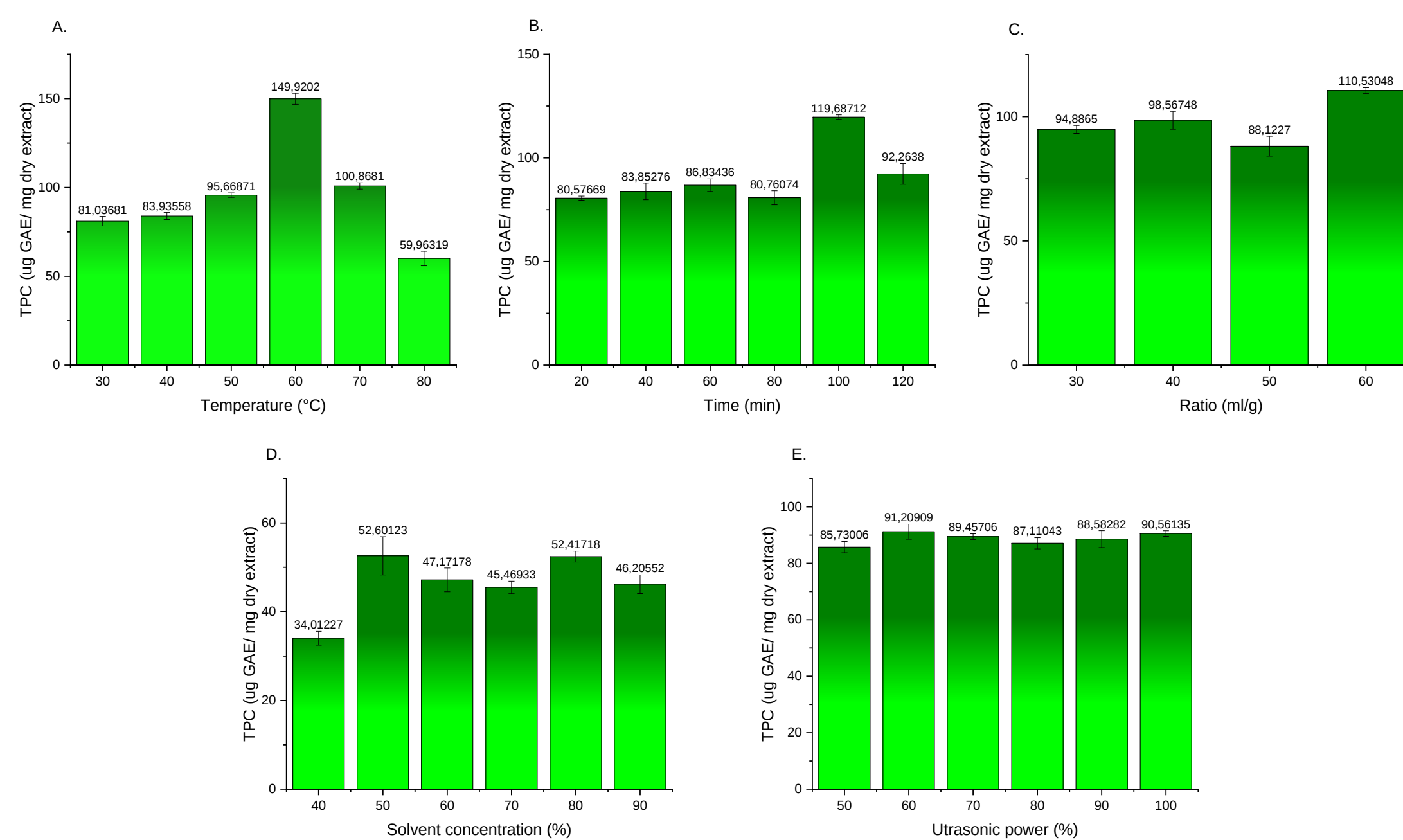


Figure. 2 Effect of extraction parameters on the total phenolic compounds (TPC)

- Figure 2 clearly shows that temperature and extraction time had a greater effect on TPC than the other parameters. Furthermore, it can be observed that temperature was the most influential factor overall.
- **The effect of extraction temperature on TPC**
- Figure 2A shows that as extraction temperature increased, TPC increased and reached a value of 149.920 ± 3.12 µg of GAE/mg dry extract at 60°C and then decreased at above 60°C.
- Temperature is a critical factor influencing the extraction rate of polyphenols. Higher extraction temperatures enhance mass transport by reducing viscosity, while also improving diffusion coefficients and the solubility of polyphenolic compounds [10] [11]. The decrease in TPC concentration at temperatures above 60 °C can be explained by the fact that phenolic compounds are heat sensitive, and beyond a certain threshold, temperature may lead to the thermodegradation of some phenolics [10].
- **The effect of extraction time on TPC**
- As shown in Figure 2B, TPC increased with the extraction time, reaching a maximum of 119.687 ± 1.09 µg GAE/mg dry extract at 100 minutes of sonication. Further increasing the time beyond this point led to a decrease in yield. Prolonged extraction may lead to the decomposition of TPC, which in turn decreases the extraction yield [12].
- **The effect of ratio on TPC**
- Figure 2C demonstrates a positive relationship between the liquid-to-solid ratio and TPC. Higher liquid-to-solid ratios increase the contact surface between the plant material and the solvent, thus increasing mass transfer [13].
- **The effect of solvent concentration on TPC**
- Maximum TPC yield was achieved when increasing ethanol concentration from 40% to 50%, but higher concentrations (90%) led to a decrease in extraction efficiency. When ethanol concentration is balanced, it facilitates extraction by lowering the dielectric constant and increasing polyphenol solubility. However, at high concentrations, it risks dehydrating plant cells, thus hindering the diffusion of phenolic compounds [13].
- **The effect of ultrasonic power on TPC**
- no significant variation in the amount of Total Phenolic Compounds was observed in the different extracts as the ultrasonic power increased (Figure 2E). The highest TPC value was 91.209 ± 2.67 µg of GAE/mg dry extract.
- A previous study on Satsuma mandarin peels (SMP) showed similar results [14]. The authors reported that using UAE at 15 °C for 20 minutes resulted in no significant variation in phenolic compound extraction when the ultrasonic power was increased from 8 to 56 W.
- Another study found only a slight increase in TPC when higher ultrasonic power was applied. The authors suggest that as ultrasonic power increased, there was a rise in the numbers of bubbles generated in the solvent during cavitation, leading to a reduction in the efficacy of ultrasound energy transmission into the medium and/or a decrease in TPC caused by ultrasonic wave with high power [15].

CONCLUSION

- This study optimized the extraction of phenolic compounds from *V. sinuatum* using a single-factor experimental approach. The determined optimal parameters were: an extraction temperature of 60 °C, a time of 100 min, a liquid-to-solid ratio of 60 mL/g, an ethanol concentration of 50%, and an ultrasonic power of 60%. The corresponding TPC under these optimal conditions was 149.920 ± 3.12 , 119.687 ± 1.09 , 110.530 ± 1.14 , 52.601 ± 4.32 , and 91.209 ± 2.67 µg GAE/mg dry extract, respectively.
- Temperature was identified as the most critical parameter governing phenolic extraction efficiency, whereas ultrasonic power demonstrated a comparatively minor influence. This findings underscore the efficacy of Ultrasonic-Assisted Extraction (UAE) as a sustainable method for recovering high-value phenolic compounds from *V. sinuatum*.

