Impact of Solvent Polarity on the Phenolic Profile of *Ceratonia siliqua Pulp A*ssessed by HPLC-UV-MS

Soumaya Atouife[1], *Jamila* Elbiyad[1], *Abdelghani* Aboukhalaf[2], *Abdellah* El Habazi[1], *Rekia* Belahsen[1]

[1]Laboratory of Anthropogenetic, Biotechnologies and Health, Research Unit on Nutrition & Food Sciences, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco

[2]Laboratory of Biology, Faculty of Sciences, Department of Biology, Ibn Tofail University, Kenitra, Morocco.

Abstract. Carob tree (*Ceratonia siliqua*) is a nutritious and medicinal plant native to the Mediterranean basin and widely used in Morocco. The present study aimed to evaluate the impact of different extraction methods on the phenolic profile of carob pulp. Two types of extraction were applied: methanolic and aqueous. The phenolic profile was determined using HPLC coupled with UV detection and mass spectrometry within 0-15min Retention time window in order to target early eluting phenolic compounds typically abundant in carob pulp. Based on retention times and MS fragmentation, the main compounds detected were: gallic acid (m/z 169), dihydroxybenzoic acid (m/z 153), citric acid (m/z 191), malic acid (m/z 133), methyl gallate (m/z 183), and simple galloyl derivatives. Gallic acid and organic acids predominated in the aqueous extract, which had a lower signal intensity and less distinguishable phenolic structures. These findings demonstrate the significant influence of solvent polarity on the effectiveness of phenolic extraction and offer guidance for the valuation of carob-based functional constituents.

1 Introduction

Endemic species in the Mediterranean region, the carob tree (*Ceratonia Siliqua L.*) is well-known for its pods, which are high in sugars, dietary fibers, and bioactive substances. About 90% of the fruit is made up of pulp, which is valued for its high tannin and polyphenol content, particularly gallic acid, as well as its functional qualities, which include antioxidant, antidiarrheal, antitussive, and anticancer effects [1,2]. Along with other phenolic compounds in trace levels, it also contains a variety of flavonols, including kaempferol and quercetin [3]. Because of its many health benefits, and antitussive actions, carob pulp is commonly used as a natural cacoa replacement[4]. Furthermore, a number of studies have demonstrated the anti-inflammatory, anti-diabetic, and hypocholesterolemic properties of carob fibers, which are mostly due to their polyphenol content [5,6].

However, comparative data on the influence of extraction solvent polarity on phenolic compounds in carob pulp remain limited. A number of variables, such as variety, environment, and extraction procedures, affect the phenolic composition of carob. In this regard, the current work uses high-performance liquid chromatography combined with UV detection and mass spectrometry (HPLC-UV-MS) to describe the phenolic compounds of carob pulp according to the impact of the extraction solvent (water vs. methanol). This method helps determine ideal conditions for its nutritional and functional valorization and offers a better understanding of how solvent polarity influences the profile and abundance of extracted chemicals.

2 Materials and methods

2.1 Plant material

In the central Moroccan region of Béni Mellal-Khénifra, mature carob pods (*Ceratonia siliqua L.*) were gathered by hand. After being thoroughly cleaned and allowed to naturally dry in the sun, the pods were manually opened to extract the pulp from the seeds. After 48 hours of drying in an oven at 50 °C, the pulp was ground finely in an electric mill. Until additional analysis, the resultant powder was kept in the proper storage conditions.

2.2 Methanolic extraction

Approximately 20 g of pulp powder from each sample (wild and cultivated) were extracted in triplicate with 150 mL of pure methanol following the procedure of [7]. The mixtures were kept in the dark under continuous stirring at room temperature for 48 h. After filtration, the extracts were concentrated under reduced pressure using a rotary evaporator to obtain the crude methanolic extracts for analysis.

2.3 Aqueous extraction

For the aqueous extracts, 10 g of dried pulp powder from each sample were suspended in 500 mL of ultrapure water in amber glass flasks. This mass was selected to ensure a sufficient extract concentration for subsequent HPLC-UV-MS analysis while maintaining an appropriate solid-to-solvent ratio, as commonly reported in similar extraction protocols [5]. The suspensions were stirred at 40 °C and 150 rpm for 24 h, following a method adapted from [8]. A gentle agitation step was maintained for an additional 12 h. The extracts were then filtered and centrifuged at 3000 rpm for 15 min. The supernatant was frozen at −24 °C for 24 h and subsequently lyophilized. The dried extracts were stored protected from light until analysis.

2.4 HPLC-UV-MS

A C18 column (250 × 4.6 mm, 5 µm) with a 15-min gradient of water/formic acid (A) and acetonitrile/formic acid (B) at a flow rate of 0.8–1.0 mL/min was used for HPLC-UV-MS studies. The UV detection setting was 280 nm. Over m/z 100–1000, mass spectra were obtained in negative ESI mode. Using retention time, UV spectra, and MS fragmentation, peaks found between 0 and 15 minutes were tentatively identified in comparison with carob phenolic data from the literature.

3 Results

HPLC–UV–MS was used to assess the phenolic content of carob pulp extracts within a 0-15 minute retention time range, which was selected to cover the elution of the main low- and medium-polarity phenolic compounds while ensuring adequate peak resolution and a stable baseline. Compared to the water extract (Figure 2), the methanolic extract had a richer chromatographic pattern (Figure 1), with more peaks and a stronger signal. Although the amount of early-eluting chemicals varied significantly between solvents, they were predominant in both extracts.

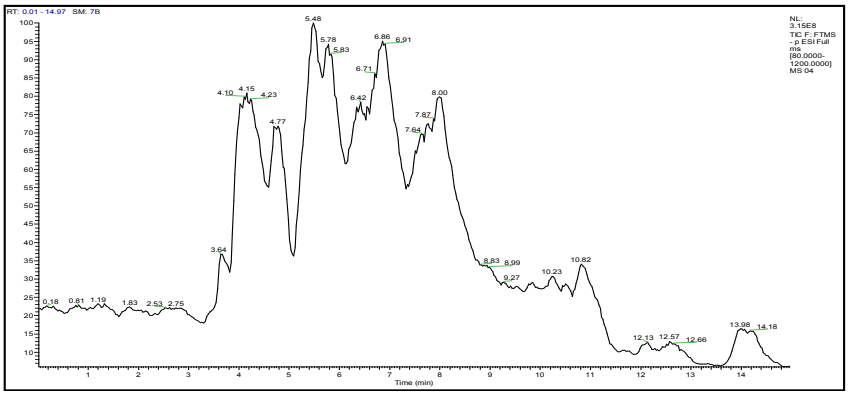

Fig. 1. HPLC–UV chromatogram of the methanolic extract of carob pulp *(Ceratonia siliqua L.)*.

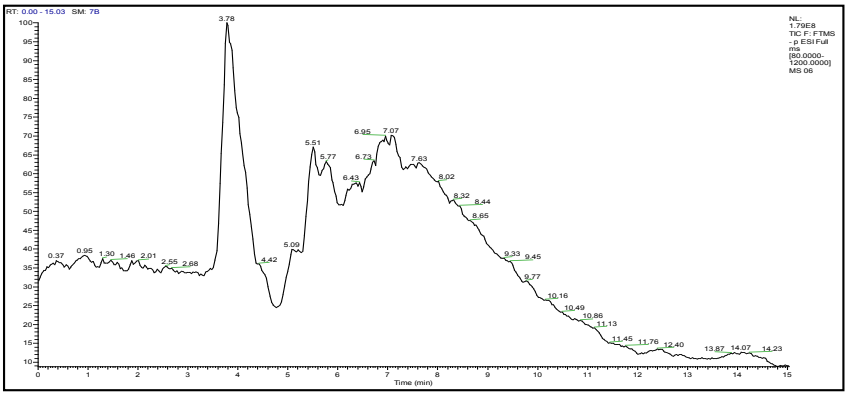

Fig. 2. HPLC–UV chromatogram of the aqueous extract of carob pulp *(Ceratonia siliqua L.)*..

Based on their UV-Vis patterns and MS fragmentation, the peaks in the 0–15 min window were tentatively assigned primarily to highly polar phenolic acids and simple galloyl derivatives, which is consistent with previously reported phenolic profiles of carob pulp [9–11]. Gallic acid and structurally similar benzoic acid derivatives were the main causes of the early-eluting peaks (0–6 min) in both extracts. Spectral fingerprints corresponding with low-molecular-weight phenolic acids and simple organic acids, which are naturally prevalent in carob pulp, were displayed by peaks that appeared at relatively longer retention durations (7–11 min). A few wider peaks found at the end of the run (12–15 min) could indicate the existence of more complex phenolic compounds or early-eluting tannin fragments.

The methanolic extract showed a richer and more intense chromatographic pattern, which enhanced the recovery of medium-polarity phenolic chemicals, as previously observed in comparative extraction studies[12]. On the other hand, despite having a decreased overall peak strength, the aqueous extract preserved a significant portion of highly hydrophilic phenolic acids, as evidenced by the existence of many early-eluting peaks.
This study's qualitative phenolic profile is in line with earlier HPLC-MS analyses that found gallic acid, benzoic acid derivatives, simple galloyl compounds, and minor organic acids to be the most prevalent early-eluting components of carob pulp [3,9,13]. The short gradient used here is appropriate for resolving highly polar and early-eluting phenolics that naturally predominate in carob pulp extracts, as these compounds predominated within the 0–15 min analytical window. This window did not include less polar flavanols and bigger tannin structures, which are usually seen at longer retention durations in prolonged HPLC tests [13]. Organic solvents (or hydro-alcoholic combinations) are more effective than water at extracting carob polyphenols, as seen by the increased complexity and signal intensity seen in the methanolic extract. This supports earlier authors' findings that the best method for optimizing the recovery of phenolic antioxidants from carob pulp is extraction based on methanol or ethanol [11,12]. Therefore, whereas water extraction might be more appropriate for conventional or "clean label" applications, the current results support the use of methanolic extraction for analytical purposes and for the synthesis of phenolic-rich carob constituents.

4 Conclusion

This study showed that the phenolic profile of *Ceratonia siliqua L.* pulp is significantly influenced by solvent polarity. While the aqueous extract was primarily made up of highly polar phenolic acids, the methanolic extraction produced a richer and more varied chromatographic pattern that allowed the detection of gallic acid, dihydroxybenzoic acid, methyl gallate, catechin, epicatechin, and early quercetin/myricetin derivatives. These results indicate carob's potential for nutritional and functional valorization and offer helpful insight into the ideal circumstances for carob phenolic extraction. In order to strengthen the connections between composition and functionality, future research should concentrate on expanding the chromatographic window to identify bigger tannin structures, measuring the discovered compounds using reliable standards, and assessing each extract's biological activity.

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