Optimization of ultrasound-assisted extraction of *Pistacia lentiscus* L. leaves in a green way to obtain the highest content of polyphenols using a response surface methodology

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Background

Pistacia lentiscus L. is an evergreen shrub, widespread over the Mediterranean basin [1]. Its leaves are rich in polyphenols, including gallotannins and flavonoids. These two main classes of compounds have several industrial and commercial applications and for example, they are both used in the food and nutraceutical industry as flavoring agents and beverages additives [2].

Furthermore, tannins are widely applied in the leather manufacturing [3]. Besides these applications, polyphenolic plant extracts, are especially utilized in cosmetic, and pharmaceutical products [4].

Thus, obtaining extracts enriched in different classes of these compounds is of high interest [5].

This work aimed to evaluate the effect of different variables on the ultrasound-assisted extraction (UAE) conditions of *P. lentiscus* L. leaves using a first-step screening design and to optimize the extraction process, using a Box–Behnken design, in order to obtain extracts with higher amounts of different classes of polyphenols (quantified by high performance liquid chromatography coupled to diode array detection, HPLC-DAD). In addition, we performed a characterization of the major compounds present in the extract with the highest content in polyphenols using liquid chromatography-mass spectrometry (LC-MS/MS).

Material and Methods

Pistacia lentiscus leaves

Samples were grinded with liquid nitrogen and weighted (300 mg)

UAE extraction

Screening design

x_1 Temperature; X_2 Time; X_3 Solvent ratio; X_4 Ethanol fraction

Optimization Box-Behnken design 3^3

x_1 Ethanol fraction; x_2 Solvent ratio; x_3 Temperature

HPLC-DAD and LC-MS/MS for the quaantification and characterization

Results

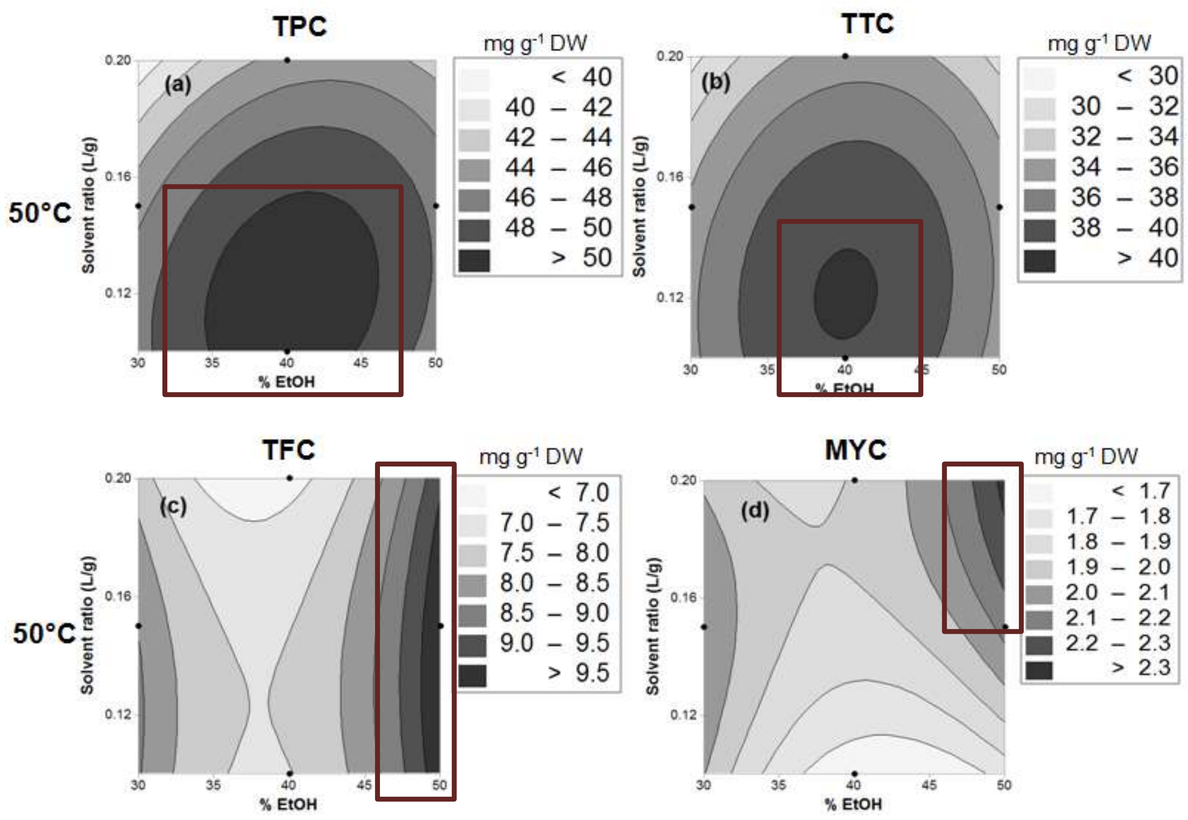

Figure 2. Response surface (contour plots) of the best conditions for TPC (a), TTC (b) TFC (c) and MYC (d).

Applying the BBD with a fixed extraction time of 15 minutes and at a temperature of 50°C, we found two different optimized extraction conditions for different classes of polyphenolic compounds. The optimal conditions proposed by the model for total polyphenols content (TPC) (**Fig. 2a**) were obtained using 40% ethanol in a ratio of 0.12 L g^{-1} resulting in 51.3 ± 1.8 mg g^{-1} DW. The total tannin content (TTC) **(Fig. 2b**) showed very similar responses surfaces to TPC. Indeed, the optimal conditions for maximize the content of tannins are the same for TPC, yielding 40.2 ± 1.4 mg g^{-1} DW. For total flavonoid content (TFC) (**Fig. 2c**) and myricitrin (MYC) (**Fig. 2d**), a different percentage of ethanol (50%) and a solvent ratio of 0.13 L g^{-1} should be used. Under these conditions were obtained 2.6 ± 0.19 mg g^{-1} DW of MYC and 10.2 ± 0.8 mg g^{-1} DW of TFC, respectively.

Three main classes of polyphenolic compounds were found with the LC/MS-MS. At 280 nm, gallic acid derivatives (**peaks 1 and 2**), digalloyl and trigalloyil quinic acid isomers (**peaks 3–7**), as well as two tetragalloyl quinic acid (**peaks 8–9**), here firstly reported in lentisk leaves were detected. At 350 nm, ten flavonoids were detected: three myricetin derivatives (**peaks 10, 11, 14**), six quercetin derivatives (**12, 13, 15-18**) and one kaempferol derivative (**19**). Myricitrin (**peak 14**) was the most abundant flavonoid detected.

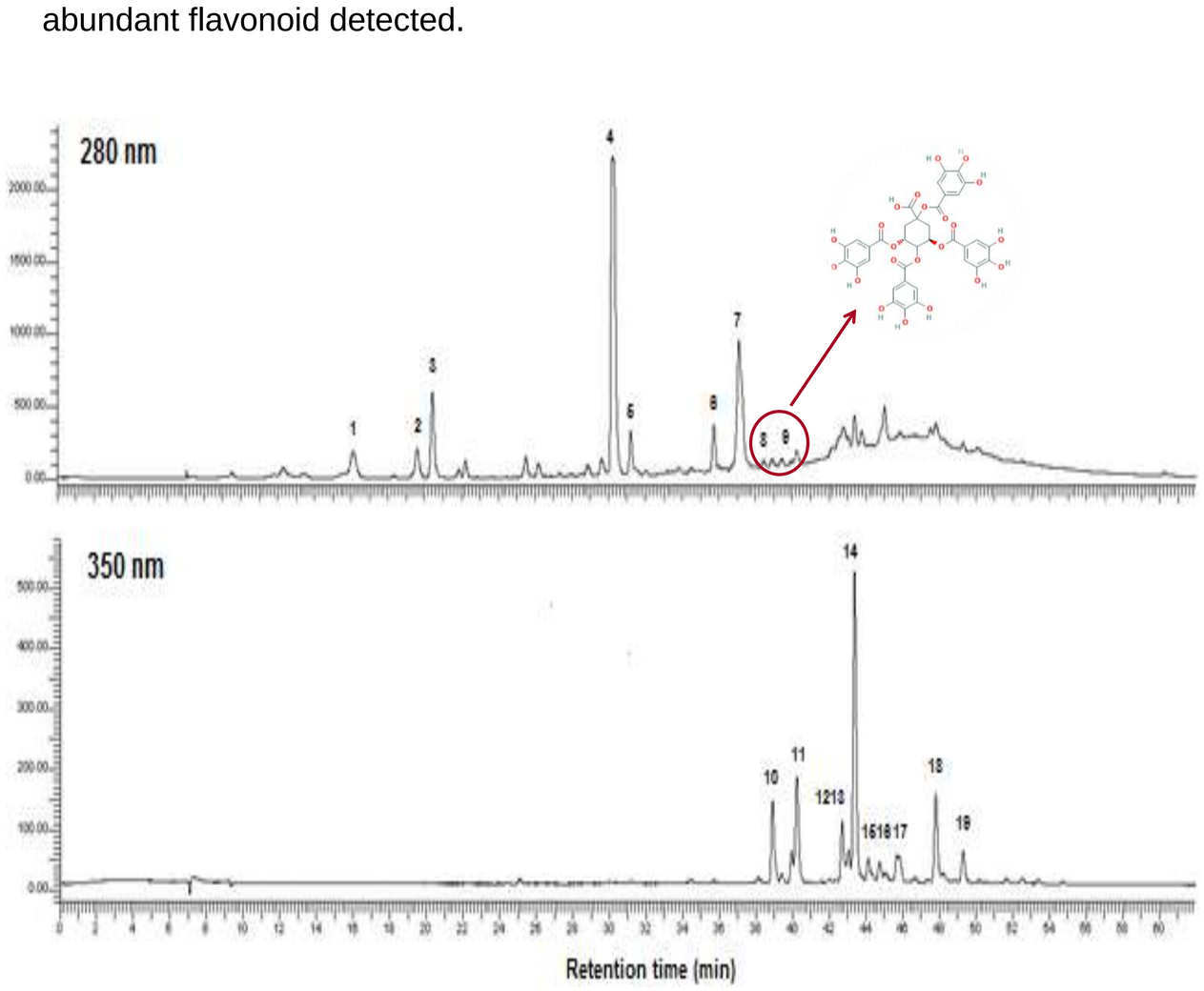

Figure 1. Chromatograms of *P. lentiscus* leaves extracts obtained using the optimized extraction conditions acquired at 280 nm (top) and 350 nm (bottom).

Conclusion

We found the optimal UAE conditions to obtain higher amounts of different polyphenolic classes from *P. lentiscus* L. leaves in a greener way, if compared to conventional extraction methods. The extraction conditions optimized here are suitable for further large-scale and industrial applications, since they apply a green solvent (ethanol: water) in low quantity, for a short time and using moderate temperatures. Furthermore, this work brings novelty in the characterization of *P. lentiscus* L. leaf extracts, putatively identifying for the first time the presence of two tetragalloyllquinic acid derivatives .

References

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