Fractionation of rosemary solid residue from steam distillation: the relationship between phenolic profile and biological activities

I. Ziani[1], M. Genva[2], H. Bouakline [1], O. Sayah[1], ML. Fauconnier [2], A. EL Bachiri [2]

[1] Physical Chemistry of Natural Substances and Process Research Team, Laboratory of Applied Chemistry and Environment, Faculty of Sciences, chemistry department, Mohammed First University, 60000 Oujda, Morocco

[2] Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio Tech, University of Liège

Introduction

Every year, the agricultural industry generates numerous hydrodistillation residues, rich in energy and essential for a sustainable bioeconomy. However, their accumulation leads to environmental problems and a potential loss of elements that can promote the production of bioactive phenolic compounds [1]. In this context, the yield of essential oil obtained by the two extraction methods (hydrodistillation and steam distillation) only reaches a maximum of 2.5% of the essential oil, generating various types of solid residues (10-20 kilotonnes/year) that could create an environmental problem if not properly managed. The biological activity of rosemary residues has not been extensively studied, and the plant's crude phenolic extracts often contain impurities [2]. Purification or fractionation of these extracts can improve phytochemical and pharmacological characterization. Assessing the biological properties of fractions containing predominant phenolic groups is crucial for selecting plants of high pharmacological interest, or for developing functional food ingredients with health-protective properties.

Experimental protocol

Fractionation of rosemary solid residue remaining after steam distillation

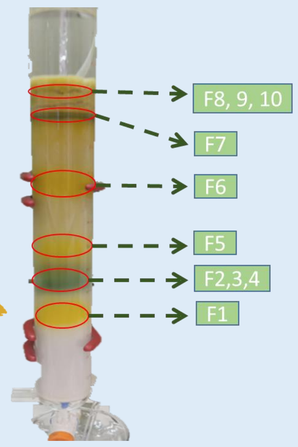
The identification of the separated molecules by high-performance liquid chromatography-photodiode array detector (HPLC-DAD)

The biological activities of the various fractions, notably antioxidant, Anti-tyrosine and antimicrobial

Separation of phenolic compounds using silica gel column chromatography

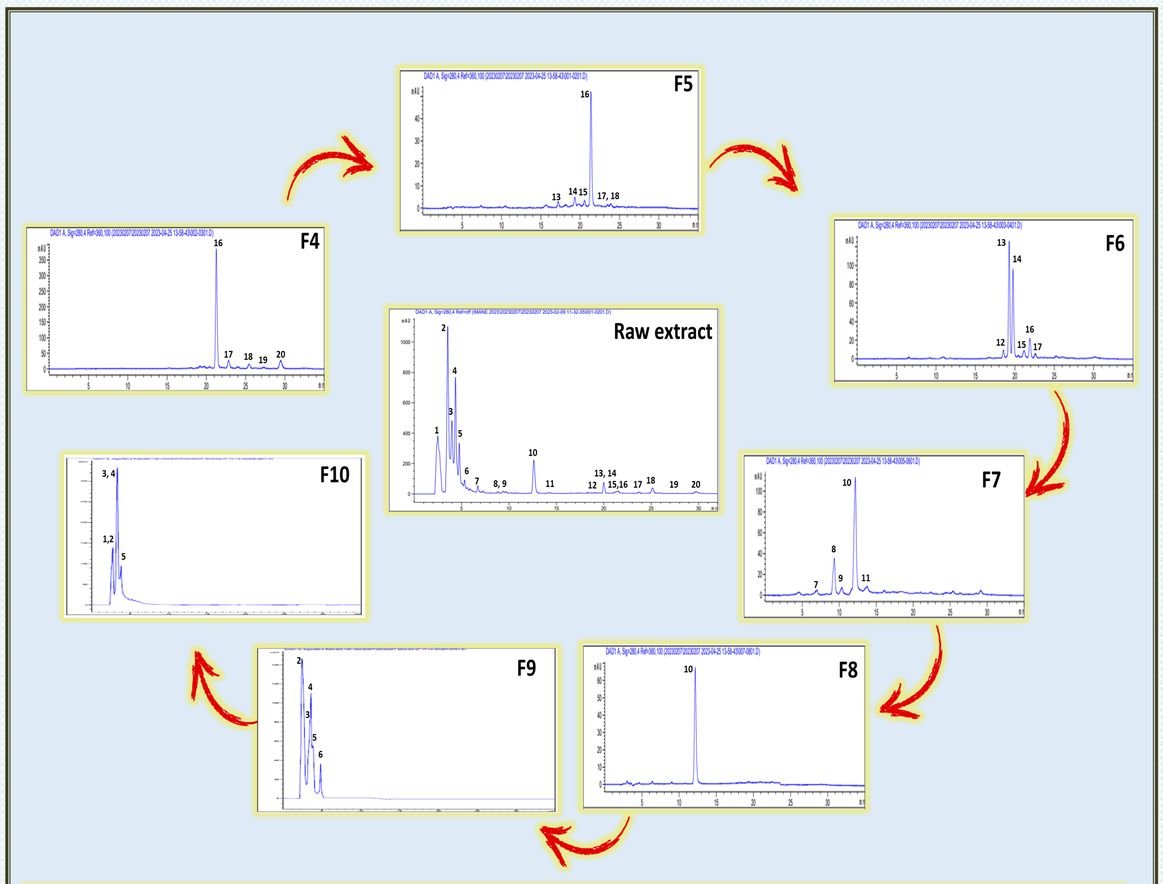
Rosemary by-product from steam distillation

Methanol extraction



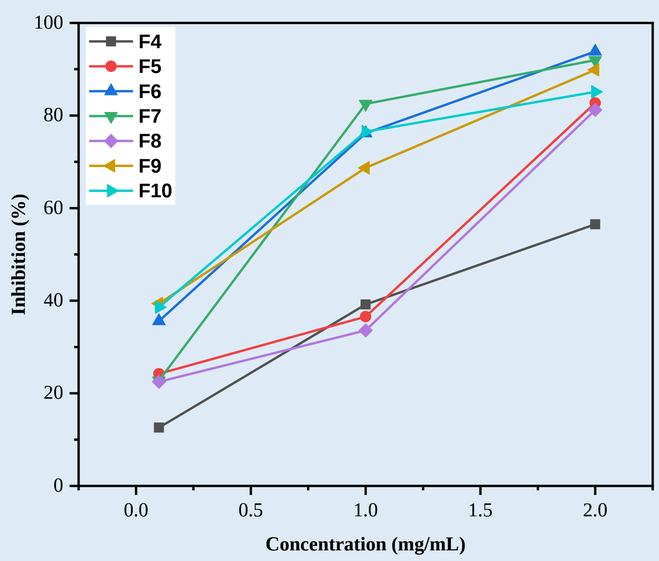
Silica gel separation

High-performance liquid chromatography (HPLC-DAD) analysis of phenolic compounds

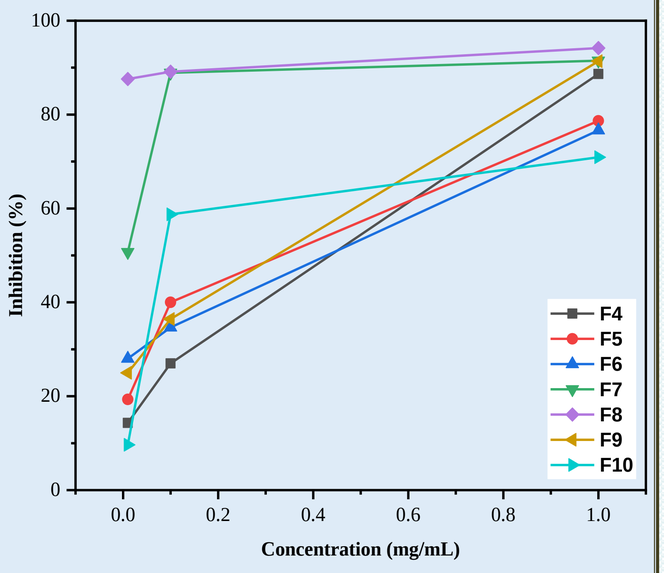


1; Gallic acid, **2**; 4-Hydroxybenzoic acid, **3**; 3-Hydroxyflavine, **4**; Caffeic acid, **5**; Rosmarinic acid, **6**; 2-Hydroxyl cinnamic acid, **7**; Kaempherol, **8**; Apigenin, **11**; Flavone, **12**; Flavonone, **13**; Chalcon, **14**; Carnosol, **20**; Carnosic acid

Biological activities

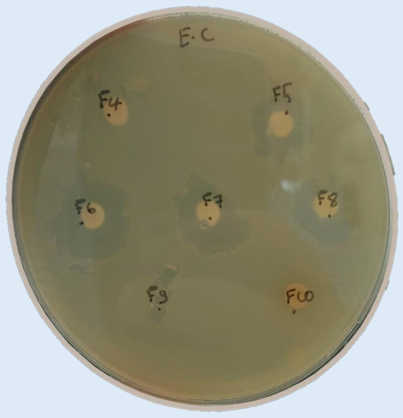


Antioxidant activity of fractions of rosemary solid residues using DPPH

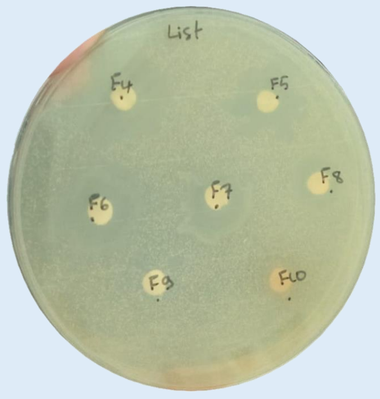


The anti-tyrosinase activity of fractions of rosemary solid residues

❖Both activities show significant differences between the separate molecules for which fractions F7 and F8 are the most active as antioxidant and anti-tyrosinase activities, respectively. Both fractions had the same main molecule, so we can assume that it probably comes from this phenolic compound.



Escherichia coli Gram −



Listeria innocua Gram +

Geotrichum sp.

❖The antibacterial activity of the compounds separated from rosemary solid residue changes significantly with fractionation, for which inhibition bacterial diameter measurements range from 13.5 to 15.4 mm for *Listeria innocua* and *Escherichia coli*, respectively. The same is true for inhibition of the mold *Geotrichum.s p*, for which fractions from F5 to F8 are the most active, and especially F8 with 16.6 mm inhibition should be the highest zone.

Conclusions

Phytochemical analysis of the phenolic fractions of rosemary solid residues, using a validated analytical method, with further evaluation of their free radical scavenging, tyrosinase inhibition and antimicrobial activities, revealed great phytochemical diversity and highlighted the predominant phenols in rosemary residues. Among them, kaempherol, apigenin, flavone, flavonone, chalcon and carnosol can be considered as markers of antioxidant, anti-tyrosinase and antimicrobial activities of rosemary solid residue fractions. These compounds could be useful as promising chemoactive agents for new phytopharmaceutical and nutraceutical preparations.

References

[1] I. Ziani et al., J. Food Meas. Charact., no. 0123456789, 2022, doi: 10.1007/s11694-022-01722-6.

[2] A. V. Ferlemi et al., Chem. Biol. Interact., vol. 237, pp. 47–57, 2015, doi: 10.1016/j.cbi.2015.04.013.