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HPLC-QTOF-MS^2-based rapid screening of secondary metabolites from horehound-*Marrubium vulgare* (Lamiaceae) leaves

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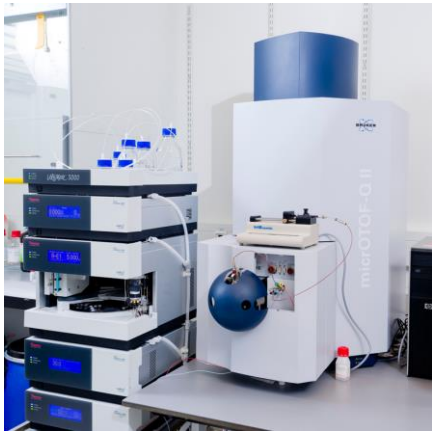
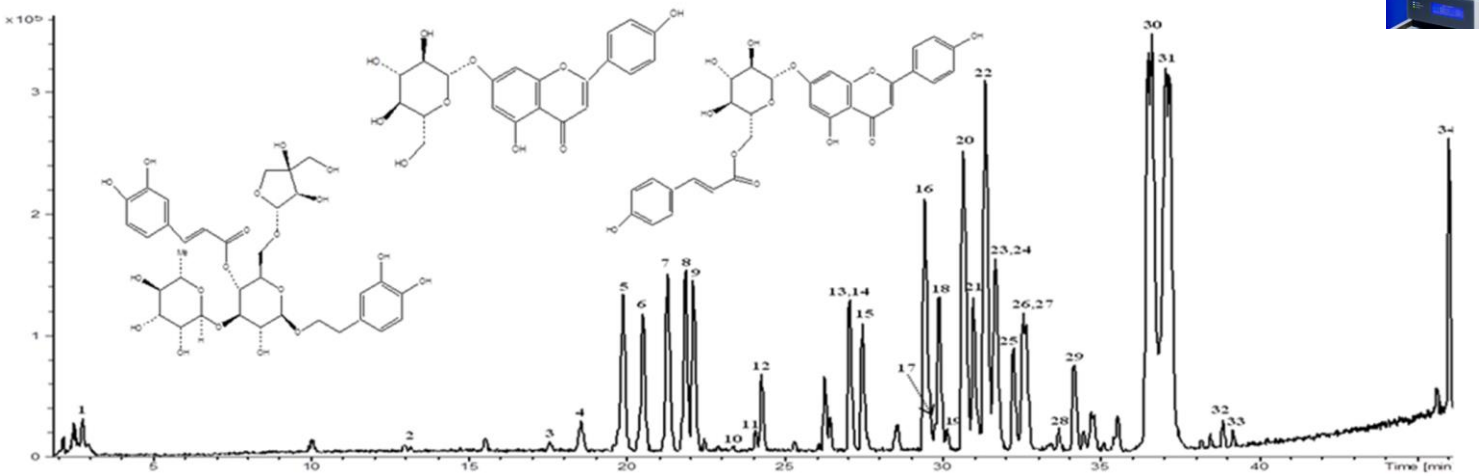
Secondary metabolites from *Marrubium vulgare* leaves

Graphical Abstract





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Abstract:

The leaves of *Marrubium vulgare* were investigated for their antioxidant activity and phytochemical composition by using high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS^2). Total contents of phenols, flavonoids, and tannins have also been determined by colorimetric dosage. The HPLC-MS^2 analysis revealed the presence of about 30 compounds of various families, of which, 15 compounds were tentatively characterized for the first time. Methanol extract was shown to contain more flavonoids and tannins. The ability of the methanol extract to scavenge H_2O_2 was found considerable (~60%). The best whole antioxidant capacity was demonstrated in the methanol leaf extract, indicating its use as a promising source of natural antioxidants for food and pharmaceutical industries, also, in hindrance of oxidative-stress-related diseases.

Keywords: *Marrubium vulgare*; HPLC-MS^2; Phenylethanoid derivatives; Phenolics; Antioxidant activities

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Materials and Methods

- The leaves of *M. vulgare* were harvested in 2009, in remote areas in the suburbs of Souk Elbatel (Bajaia, Algeria).
- Fresh leaves were air-dried in shade at room temperature. After drying, ground to a fine powder (diameter < 250 µm) using an electric mill, thereafter, powder was exhaustively extracted by maceration with methanol, at room temperature for 24 h.
- Acetone extraction solvent was used for the antioxidant activity determination assays.
- Analyses were made using an Agilent 1200 Series RRLC system (Agilent Technologies). Zorbax Eclipse Plus C18 (150mm×4.6 mm, 1.8µm) column has been used. The UHPLC was coupled to a microTOF-Q mass spectrometer (Bruker Daltonics). For the antioxidant activity assays, a spectrophotometer (UV mini 1240, SHIMADZU) was used.

Materials and Methods

- The total phenolic content was assayed using the Folin-Ciocalteu reagent, following the Singleton and Rossi method (1965).
- The flavonoid content was determined using the method of Huang et al. (2004).
- Total tannins were estimated according to the protocol developed by Hagerman and Butler (1978) on the basis of their precipitation by a protein, bovine serum albumin (BSA).
- The DPPH (1,1-diphenyl-2-picrilhydrazyl) radical scavenging activity of the plant extract was determined using the method proposed by Katalinic et al. (2006).
- The scavenging capacity of M. vulgare leaves extract on hydrogen peroxide was determined according to the method described by Ruch et al. (1989).

Introduction

- *M. vulgare* is traditionally used alone or combined with other herbs to treat coughs, bronchitis, and colds. The leaves and flowering stems have tonic, aromatic, stimulant, expectorant, diaphoretic, and diuretic properties. It has also vasorelaxant, antioedematogenic, antihepatotoxic, and antihypertensive activities. It is traditionally used for its antioxidant, antibacterial, analgesic, antispasmodic, hypoglycemic, and hypolipidemic effects.
- *Recently, we have demonstrated the potential role of this plant in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities.
- Due to the wide use of this plant to treat many diseases and as a part of continuation of our studies, we have explored its chemical composition to determine its potential role in terms of the antioxidant activity.

***Amessis-Ouchemoukh, Abu-Reidah, Quirantes-Piné, Rodríguez-Pérez, Madani, Segura-Carretero, Fernández-Gutiérrez, 2014.**

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Results and discussion

- *M. vulgare* leaf extracts were found to contain valued contents of total phenolics, flavonoids, and tannins. Notably, the methanol extract contained more flavonoids and tannins than did the acetone extract, while the amount of total phenolic compounds presented no significant differences (p>0.05). These results suggest that methanol is a good solvent for extracting phenolic compounds.

- The variation in the amounts of phenolics in the methanol and acetone extracts could be attributed to the solubility of phenolic compounds. Moreover, it has been reported that the solubility of phenolic compounds is governed by the type of solvent used, the degree of polymerization of the phenolics, as well as by the interaction of the phenolics with other food constituents and the formation of insoluble complexes.

- *M. vulgare* leaves were found to contain higher amounts of flavonoids and total phenolics than *M. deserti* belonging to the same genus, and lower amounts of phenolics compared with those found in the same species by others. In terms of tannins, the amounts were found to be higher in the methanolic extract. Several studies have reported the presence of tannins in this plant elsewhere.

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Results and discussion

- The percentage of DPPH scavenging activity range from 51.90 to 97.15%. All the samples presented more than 50% of radical inhibition; these results revealed that considerable antiradical components were extracted from the plant leaves. Both acetone and methanol plant extracts have inhibited the DPPH radical and no significant differences between them (p>0.05) were shown. These results could be attributed to the richness of the extracts in phenylethanoids and flavonoids determined in this study.

- The correlation level between the phenolic content and the DPPH·scavenging activity was extremely significant (r=0.53) (Table 4). *M. vulgare* was also found to contain phenylethanoid glycosides. These were later known to have significant antioxidant properties with this test, which indicated their ability to efficiently scavenge free radicals (Calis et al., 1999; Es-Safi et al., 2005). The synergy of these components with one another and/or other components present in an extract may contribute to the overall antioxidant activity observed.

Results and discussion

- The ability of methanol extract of *M. vulgare* to scavenge H_2O_2 was very high (59.73%), while acetone extract was not determined due to the appearance of turbidity under the test conditions. BHA and vitamin E showed the strongest scavenging activity, 66.10 and 65.38 %, respectively.

- The results showed that methanol extract of *M. vulgare* was very high compared to rutin and nearly equal to that of BHA and vitamin E. These results could be explained by the presence of phenolic compounds, which have the capacity to eliminate radicals.

- The total antioxidant capacity exhibited by the methanol leaf extract was higher than the acetone extract which implies that methanol has extracted more compounds with more possible antioxidant activity. A highly significant correlation coefficient was found between the amounts of flavonoids (r=0.99), phenolic compounds (r=0.63), and the total antioxidant capacity. In addition, crude extracts were known to contain different components and synergy between them may contribute to the overall observed antioxidant activity.

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Conclusions

- In this work, the use of UHPLC-ESI-QTOF-MS has proved to be a useful tool to identify new phenolic and terpenoid derivatives.
- The leaves of *M. vulgare* were found to have a wide array of phytochemical compounds such as flavonoids, phenylethanoids, and terpenoids.
- The colorimetric dosage has shown the methanol extract to contain more flavonoids and tannins. In the antioxidant activity, the percentage of DPPH radical-scavenging activity ranged from 51.90 to 97.15%.
- Total antioxidant capacity exhibited by methanol leaf extract was higher than that of acetone.
- Obtained results highlight the importance of this plant as a promising source of natural antioxidants for food preservation and oxidative stress-related disease prevention or/and delay together with its use in the industries related to pharmaceutical and functional ingredients.

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