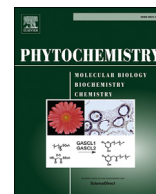




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Review

Separation of phytochemicals from *Helichrysum italicum*: An analysis of different isolation techniques and biological activity of prepared extracts

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ABSTRACT

Helichrysum italicum presents a valuable source of natural bioactive compounds. In this work, a literature review of terpenes, phenolic compounds, and other less common phytochemicals from *H. italicum* with regard to application of different separation methods is presented. Data including extraction/separation methods and experimental conditions applied, obtained yields, number of identified compounds, content of different compound groups, and analytical techniques applied are shown as corresponding tables. Numerous biological activities of both isolates and individual compounds are emphasized. In addition, the data reported are discussed, and the directions for further investigations are proposed.

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1. Introduction

The genus *Helichrysum* consists of an estimated 600 species. Its name is derived from the Greek words *helios* (sun) and *chrysos* (gold). *H. italicum* (Roth) G. Don fil. (syn. *H. angustifolium* subsp. *italicum* (Roth) Briq. & Cavill.) from the family Asteraceae is commonly known as the curry plant or the everlasting plant. It grows on dry, rocky, or sandy ground around the Mediterranean. The stems are woody at the base and can reach 30–70 cm in height (Viegas et al., 2014). It is a small aromatic shrub with yellow flowers. *H. italicum* can be further divided into six subspecies distributed in different regions of the Mediterranean basin: *H. italicum* (Roth) G. Don subsp. *italicum*, native to the Mediterranean basin; *H. italicum* subsp. *microphyllum* (Willd.) Nyman, characteristic for Balearic Islands (Majorca and Dragonera), Sardinia, Corsica, Crete, and Cyprus; *H. italicum* subsp. *picardii* Franco, found in the flora of France, Italy, Portugal, and Spain; *H. italicum* subsp. *pseudolitoreum* (Fiori) Bacch, related to Argentario, Gargano, and Monut Conero; *H. italicum* subsp. *serotinum* (Boiss.) P. Fourn., found in Iberian Peninsula; and *H. italicum* subsp. *siculum* (Jord. & Fourr.) Galbany, found in Sicily (Viegas et al., 2014). The relationships between the location and the genetic data among some subspecies have already been reported (Galbany-Casalas et al., 2011).

Isolates of *H. italicum* consist of many different compound classes, among which the most common are terpenes and phenolic compounds. Because of the variety of specialized metabolites, *H. italicum* has been reported to possess a wide range of biological activities. Viegas et al. (2014) in their review article compared reports on the traditional use of *H. italicum* with those on the activity of single isolated compounds stating the abundance of literature data. Traditional use of this plant includes the application for the treatment of allergies, colds, cough, skin, liver and gallbladder disorders, inflammation, infections, and sleeplessness. It was one of the main topics of Lourens et al.'s (2008) review article in which biological activity and phytochemistry of South African *Helichrysum* species were also investigated. The results presented by Guinoiseau et al. (2013) referred to the classification of the main biological activities of *H. italicum* essential oils and extracts into five groups: antimicrobial, anti-inflammatory, anti-viral, antioxidant, and anti-larvicidal activities. The emphasis was given to the correlation between the most common individual compounds and their reported activities.

This review article is an attempt to classify and critically examine the literature data on biologically active compounds isolated from *H. italicum* with regard to the particular experimental procedure of isolation. According to the results of application of distinct isolation procedures presented in literature, special attention was given to terpenes and phenolic compounds, followed by other less significant phytochemicals. The analyzed isolation procedures were hydro- and steam distillation and supercritical CO₂ extraction, as the favorable procedures for the separation of terpenes, and extraction with organic solvents, which is mainly used for isolation of phenolic compounds. The reported results based on the isolation procedures are presented as tables. In addition, the biological activities of extracts and pure compounds are also given but to the extent that ensures nonrepetition of the presented data in the abovementioned review articles (Guinoiseau et al., 2013; Lourens et al., 2008; Viegas et al., 2014).

2. Terpenes of *H. italicum*

Terpenes, one of the most significant specialized metabolites present in *H. italicum*, possess numerous biological activities, both individually or as a part of plant isolates. Among them, the greatest contribution to the composition and biological activity of

H. italicum isolates belongs to mono- and sesquiterpenes. As mentioned, the majority of reported results indicated that hydro-distillation, including steam distillation, followed by supercritical CO₂ extraction and, in a few cases, extraction with organic solvents were used for the separation of terpenes from *H. italicum*. Therefore, this section mainly focuses on terpene constituents of *H. italicum* essential oils, obtained by distillation processes, and supercritical extracts, emphasizing the data of isolation/extraction procedures and biological activities of extracts/pure compounds. In addition, special attention was given to the most significant representatives of *H. italicum* terpenes.

2.1. Distillation of *H. italicum* aerial parts

Distillation of *H. italicum* aerial parts is the most commonly used technique for obtaining the essential oils, according to the large amount of literature data. The majority of literature data regarding *H. italicum* essential oils obtained by distillation referred to the two main subspecies: *H. italicum* (Roth) G. Don subsp. *italicum* and *H. italicum* subsp. *microphyllum* (Willd.) Nyman. Two distinct types of distillation are presented in the literature: hydrodistillation performed in a Clevenger-type apparatus and steam distillation performed in a spring-type apparatus.

2.1.1. Hydrodistillation of *H. italicum* aerial parts

Studies reporting hydrodistillation of *H. italicum* essential oils in a Clevenger-type apparatus are numerous. According to the literature data, the applied time for distillation varied from 1 to 5 h, and the obtained yields were in the range of 0.02–0.78%. The most common methods for analysis and identification of essential oil components were GC-FID and GC-MS. Reported experimental conditions and results in terms of number and overall percentage of the identified compounds and the main components of the compounds are given in Table 1. Some authors reported the contents of different compound groups in essential oils, and these data are presented in Table 2.

Activities of essential oils obtained by hydrodistillation are diverse. Leonardi et al. (2013) investigated the composition of 21 essential oil samples isolated from *H. italicum* (Roth) G. Don subsp. *italicum* collected at 7 locations of Elba Island (Tuscany, Italy), characterized by different soil types during three different periods (January, May, and October 2010). The results of applied statistical analysis showed a difference in the composition of the essential oils mainly because of the environment where the plant grew, and, in particular, to the soil type. Essential oils isolated from samples collected in locations characterized by intrusive igneous rocks were rich in α -humulene, γ -muurolene, β -caryophyllene, cis- α -bergamotene, eudesm-5-en-11-ol, eudesmol isomers, and neryl acetate. Essential oils of sandstone soil samples were characterized by the presence of α - and β -pinene, camphene, γ -terpinene, 1,8-cineole, α -terpineol, borneol, nerol, and linalool. Furthermore, essential oils of samples growing on quaternary deposits were rich in eudesm-5-en-11-ol, viridiflorol, nerol and its acetic acid a propanoic acid esters, δ -selinene, ar-curcumen, and limonene. Finally, essential oils of samples collected from serpentine soils were characterized by the presence of neryl acetate, neryl propanoate, ar-curcumen, italicene, and limonene. Similar to Leonardi et al. (2013), Paolini et al. (2006) studied essential oils of 11 samples of *H. italicum* (Roth) G. Don subsp. *italicum* collected from six Tuscan archipelago islands that showed similarities in the main composition with that of Corsican, Sardinian, and North American *H. italicum* subsp. *microphyllum* essential oils. Satta et al. (1999) studied the chemical composition of essential oils of *H. italicum* subsp. *microphyllum* (Willd.) Nyman collected at different locations in Sardinia, at altitudes between 100 and 900 m. Essential oils obtained at altitudes

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