

Exploitation of ethnopharmacological data towards the discovery of novel wound healing agents

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Introduction

Skin disease treatment is a global concern, especially in the case of chronic wounds where despite scientific progress, their comprehensive treatment remains still a challenge (Posnett et al, 2009). The proven wound healing properties of several traditionally used plant species (eg *Alkana tinctoria*, *Pistacia lentiscus*) is a strong evidence of their dynamics in wound management. Due to their multitargeted biological activities, natural products are increasingly gaining scientific interest as the perfect candidates for dealing with the multifunctional and complex process of wound healing pathophysiology. Traditional practices and ethnopharmacological information regarding the use of plants for treating several diseases by mankind, have often been used as a useful tool for the discovery of potent and novel therapeutical agents (Fabricant et al., 2001).

To this direction, the aim of our study was the discovery of new natural agents with potent wound healing activity, through the utilization of ethnopharmacological data and the application of analytical and biological evaluations. An extensive recording of ethnopharmacological data referring to the use herbs for the treatment of several skin conditions was realized, using ancient medical texts and ethnobotanical / ethnopharmacological studies conducted in Greece. The area of focus was mostly the region of Crete, due to high biodiversity and long-term and frequent use of herbs by its inhabitants. The selected plant species were extracted in order to evaluate their chemical content as well as their wound healing capacity by assessing their phenolic / flavonoid content, antioxidant activities and collagenase inhibition properties related to skin elasticity and

moisture. Finally, the most promising extract was subjected to further phytochemical analysis.

Material and methods

The recording of the ethnopharmacological data was realized through: a) an extensive literature research via Scopus, PubMed, Reaxys, Google scholar etc., regarding the traditional uses of herbs in the wider region of Greece for the treatment of wounds. Various keywords were used such as “plants”, “herbs”, “traditional remedies”, “wounds”, “burns”, “scars” and similar skin diseases and also “ethnobotany”, “ethnopharmacology”, “ethnomedicine”, “Greece”, “Crete” etc. 216 plant species were recorded. b) an extensive study of Dioscorides’ manuscript “De Materia Medica” extracting information about 112 plants used for their healing ability along with the way of their preparation for their respective indication of use. The majority of the recorded plants were answered in both lists.

The selected species were collected following the good botanical practices and subsequently they were extracted using the ultrasound assistant extraction (UAE). The pulverized plant material was extracted using dichloromethane, methanol and methanol:water 50:50, successively. Their chemical profile was determined using High Performance Thin Layer Chromatography (HPTLC) and High Performance Liquid Chromatography coupled with a Diode Array Detector (HPLC-DAD).

The total phenolic content (TPC) and the total flavonoid content (TFC) of the plant extracts, were determined using the Folin-Ciocalteu method (Singleton et al., 1999) and the aluminium chloride colorimetric method, respectively (Karapetsas et al., 2019). Their

antioxidant activity was evaluated through the DPPH assay (Stagos et al., 2012) at 200 µg/mL concentration and IC50 values were calculated for the most potent extracts. The anti-collagenase activity of crude extracts, was determined based on a previously described spectrofluorimetric method (Hartmann et al., 2015) with slight modifications. Extracts were evaluated at concentrations of 100 and 50 µg/mL and for the most potent extracts IC50 values were calculated.

Centrifugal Partition Chromatography (CPC) was performed for the fractionation of the most promising extract using a stepwise gradient elution-extrusion methodology and classic chromatographic techniques (Gel exclusion chromatography, HPTLC preparative) were used for the isolation of the secondary metabolites.

Results and discussion

Taking into consideration that the area of Crete was the focus of the current study, we emphasized on species characteristic or endemic of the Cretan flora such as *Cistus creticus* or *Origanum dictamnus*, leading us to the selection of 10 species from the genus *Asphodeline*, *Dittrichia*, *Hypericum*, *Origanum* and *Cistus*, for further evaluation.

The chemical profiling of the extracts revealed the presence of several bioactive compounds, such as terpenes in the case of extracts of *Origanum* and *Cistus* species and phloroglucinol derivatives and flavonoids in extracts of *Hypericum* species, along with small qualitative differences.

As expected, methanol and methanol:water extracts demonstrated higher TPC / TFC content and antioxidant capacity compared to dichloromethane extracts. As most potent antioxidant agents were characterized the methanolic and hydroalcoholic extracts of *Cistus creticus* subsp. *creticus* L., *Cistus creticus* subsp. *eriocephalus* (Viv.) Greuter & Burdet, *Cistus salviifolius* L., *Hypericum empetrifolium* Willd., *Hypericum triquetrifolium* Turra, *Origanum dictamnus* L., and *Dittrichia viscosa* (L.) Greuter.

Regarding the collagenase inhibition assay, hydroalcoholic extracts exhibited slightly higher inhibition values than the methanolic ones. *Cistus* extracts were characterized as the most potent inhibitors with the hydroalcoholic extract of *Cistus creticus* subsp. *creticus*

L. exhibiting > 80% inhibition at 50 µg/mL concentration. Potent inhibitors were also characterized the hydroalcoholic and methanolic extracts of *Origanum*, *Dittrichia* and *Hypericum* species.

The CPC fractionation of the hydroalcohol extract of *Cistus creticus* subsp. *creticus* provided 48 fractions. A primary phytochemical screening of the obtained fractions revealed the presence of flavonoids, phenolics and tanins.

Subsequently, selected fractions were subjected to further chromatographic analyses for the isolation of the major secondary metabolites

Conclusion

Phytochemical and biological screening of 10 plant extracts was performed, resulting to the characterization of their chemical profile and wound healing potent activities. Extracts from *Cistus* species are among the most promising candidates.

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